



# THE AMERICAN JOURNAL OF TROPICAL MEDICINE

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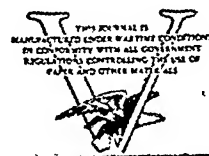
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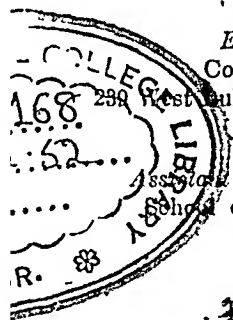
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*Editor, CHAS. F. CRAIG*  
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# A BROADER PERSPECTIVE FOR BACTERIOLOGY

## PRESIDENTIAL ADDRESS

N. PAUL HUDSON

*From the Department of Bacteriology, Ohio State University, Columbus*

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Periodically, there occurs a great event that causes men of science to pause in their work and plans, and attempt to orient their science and their thinking to the influence of the great event. Forces that bring about this self-inspection and analysis have included a change in philosophic thought, the emergence of a new scientific field, the discovery of a new tool for investigation, and the influence of an economic depression. Without entering into the controversy as to whether war is the result or the cause of scientific advancement (and both are apparently true), it cannot be denied that war is a tremendously strong factor in the stimulation of scientific advance. As a forceful event, it is causing scientific men to inspect their position and their science, to relate themselves to the powerful scientific movement of the time, and to plan for alignment with future advancement.

During this time of war, we have much more at stake than merely learning of Nature and applying our learning to the problems of war. We have, it seems, the question of whether we shall have the liberty to continue scientific inquiry without outside pressure or dictation. Under these circumstances, science must consider its association with Mankind, since it depends on human liberties for its freedom and life. For my part, I have little patience for the quibbling over the relative virtues of so-called "pure" and "applied" science. They are not distant cousins between whom we would choose, but siblings whose parents are Science and Mankind. With "pure" and "applied" having the common denominator of a scientific and social parenthood, and with Science and Mankind now in danger, there is in this crisis even less reason for us to choose between the children; how they serve to satisfy man's curiosity on the one hand, and to enrich man's life on the other is what counts.

In surveying a scientific field to determine its status and direction, it is more important to consider its perspective and the points of view of its workers, than to review merely its collection of facts about Nature. The accumulation of details

comes, not always easily or as a matter of course, but more certainly if a sound perspective and outlook have preceded. The attitude, however, is more than receptiveness to new ideas; it is a conscious effort to imagine, explore mentally, interpret, synthesize as well as analyze, reach into neighboring fields for techniques and lines of reasoning, develop open-mindedness, and to see one's science in terms of human values. Details then become means to an end and are not ends in themselves. When a science is based on a developing mental attitude, flexibility is one of its characters. Findings that seem to be facts can be regarded as of temporary value to be adjusted or discarded with the acquisition of more knowledge, when the way of thinking is not dogmatic but broad, receptive and mobile.

With these introductory remarks as a background, I should like briefly to consider the science of Bacteriology and to attempt to orient it to modern thought and the influence of the war. In so doing, I shall have occasion also to show the relation of my analysis and resulting suggestions to a cardinal principle of Tropical Medicine.

It is customary to sketch the historical background for a discussion such as this. We can then the better see why a broader perspective can be considered timely. After starting as a curiosity in the hands of Leeuwenhoek, Bacteriology made little progress for 150 years. Then through inquiries into the cause of fermentation and the truth of spontaneous generation, the scientific giants of the times, led by Pasteur, learned about the activity of microscopic forms. When the conception of fermentative action of bacteria was carried over to infectious processes by Lister and Pasteur, the whole field of microbic causation of infectious disease was disclosed. This was a most logical turn of events, which however led to confusion because of the lack of knowledge of those parasitic forms present in and on animal tissues but not primarily concerned with the particular infectious process. In a necessary and scientific solution of

this confused situation, Koch laid down the principles that guided etiologic studies by emphasizing the importance of bacterial form and pure culture.

Thus Bacteriology passed through the phases of initial curiosity, expanding microbic physiology, and restraining morphology. Not until the third decade of this century have bacteriologists recognized that a strict respect for form is not their sacred duty. Aided by the expanding study of viruses, which are recognized chiefly by their action, workers in Bacteriology now think more of what the lower forms do, than of their appearance. This about-face has been a long and painful process that has not simplified our concept of microscopic and sub-microscopic forms, but has no doubt brought us nearer to knowing the complexity of Nature's truths.

As a result of these studies of the last 75 years, man's lot has unquestionably improved. Bacteriology has contributed in no small way to "the alleviation of suffering and the prolongation of life." The yield of the soil has been bountifully increased. The gregarious habit of man has been more pleasantly satisfied by the provision of sanitary conditions and of safer and more varied foods now available from far and near. Microbiology has added to the list of industrial products used and enjoyed by Mankind.

Can we hazard a guess as to the perspective of bacteriologists of this proudly productive era? It is a dangerous generalization, since there are many kinds of persons as well as a great variety of subjects to be considered. Is my opinion correct that we have paid disproportionate attention to techniques and the development of differential media? Perhaps we have worshipped too devoutly the uncompromising goddess of taxonomy, unmindful that she imposes rigid concepts of bacterial properties that really are variable and hence should not be used for natural differentiation of bacteria. We have constantly tried to make procedures easy and determinations final, and have accepted their artificiality without question. We have looked down our test tubes and microscopes so constantly that we have become myopic in regard to the broader outlook.

I do not for a moment minimize the importance of these lines of work, which have step by step led to scientific advances and to the human advantages of which I have spoken. And, if we are critical, we should remember that the perspective

has been influenced by the contemporary scientific atmosphere of "getting the facts." Furthermore, the search for the truth about microbic and sub-microbic life has assuredly been a source of great satisfaction to the workers, whether they reaped or gleaned in the brotherly fields of pure and applied science.

However, is the present status of bacterial investigation and subjective interest all that we have the right or hope to expect? Are we justified in stopping with the accumulation of observations and the turning of certain findings to practical human use? Is the point of view to be limited to the perspective I have admittedly overdrawn?

Bacteriology has come of age and, in fact, has already given birth to at least two offspring. It has developed its own techniques, its unique principles, and its individual lines of thought. It has roughly staked out its field and forsworn its allegiance to its numerous and dubious parents. As a respected adult, Bacteriology has won the attention and admiration of other sciences. To advance with sister sciences, it must continue to grow in perception, skill and interpretation, and not be content to stay at the level of methods as the aim and end of scientific endeavor.

This, then, is how I analyze Bacteriology, in this critical time that calls for self-analysis and re-orientation in the light of the current great event. I venture to do so before this group because I recognize how much Tropical Medicine is concerned with the various phases of Bacteriology. Tropical Medicine looks to Bacteriology and allied fields for the understanding and solution of problems in infectious diseases of warm climates. With the emphasis in medicine being placed on the influence of the region on the incidence and nature of disease, and with the current conflict illustrating daily the importance of that principle of Tropical Medicine, it is pertinent that I suggest, as the basis for further bacteriologic advancement, the recognition of the role of the environment in Bacteriology.

I propose to consider the significance of environment in connection with free-living and parasitic forms.

Let me start the section concerning the free-living bacteria by making some observations, albeit bold, regarding bacterial studies *in vitro*. Test tube bacteriologists may think that they are delving into the secrets of Nature when they put through its tricks a pure culture of a single-cell

strain, isolated from the soil by a patient worker 50 years before. The unnaturalness of the situation is usually emphasized by the culture being received in the mail from a type culture collection. It arrives in the dry stage in response to a telegram, and the crumbly powder, in an atmosphere of pure nitrogen in a sealed glass tube, is laid on the laboratory table until a synthetic medium is made. Perhaps the only natural constituent intentionally included in the medium is water, to which is added a little chemically purified this and that, and the other elements we consider essential may be included in the water as contaminants or dissolved from the glass. This is the so-called "environment" which provides enough of what the bacterium needs for its structure and physiology, so that in 20 hours (by the automatic timer) a cloud appears in the test tube, to which the worker points with pride. A gram stained preparation is made, and all forms nicely appear with the same size, shape and color.

Now this is all well and good, provided the worker does not deceive himself. The finding is interesting, his curiosity may be satisfied, and perhaps the bacterial growth is of human value. I hasten to defend myself and my colleagues against the charge that this type of work is senseless; it has much sense to it; but too often it has been conducted on the basis of "getting the facts." It has and will have its place, but I plead that the worker recognize the conditions under which he does such work. He must know the artificiality of the conditions and should be willing to say, "This is the environment I set up, not the natural environment. Nature's environment is complex: the pabulum is mixed and variable; the temperature is not set at  $\pm 0.5^{\circ}\text{C}$ , sunlight is sometimes present, the water supply is irregular, and there is competition with a mixed microbic flora." When this point of view is held, the worker becomes a naturalist and he has progressed from a lower to a higher level of thought and perspective.

The fact is that bacteriologists are beginning to see that the environment is important even under artificial conditions, in respect to the effect of the medium on the regularity of the size, shape, staining, and chemical composition of the bacterium. They are understanding the lability, the variability and the responsiveness of bacteria to some of these environmental factors. Although these conditions of observation are artificial, we are learning by such studies that microbic forms are not static

and rigid but follow only broad lines of regularity influenced by factors in their surroundings.

If we turn now to the field of parasitic bacteriology, we find the argument even more pertinent. We bacteriologists do not usually make studies of parasitic forms directly from the sites of the body parasitized. If we did, we should note that the microbic forms are not as regular or as nicely arranged as our textbooks picture them. Instead of building a conception of form based on observations of bacteria *in situ*, we make cultures right off and then fall back on the artificial culture situation to build our concepts of bacterial morphology and physiology. Useful in diagnosis? Certainly, but not a true picture of Nature, because we have hastened to the use of an artificial environment and neglected the opportunity of acquiring the broader perspective of parasitism.

A basic principle of Bacteriology is pure culture study. Koch, following Henle's lead, emphasized this point in relation to causation of disease and he served the needed function of bringing order out of chaos by insisting on the exclusion of extraneous forms in studies on specific etiologic agents of infectious diseases. This dogma, good in its place and time, is so deeply entrenched in textbooks that the student usually recites the so-called Koch's postulates when questioned for evidence for the specific relation of a causative agent to a specific infectious disease. How much the student misses, particularly the medical student, if he goes no further in his concept of causation than an understanding of Koch's over-simplified enunciation! The breadth of perspective is the measure of our advance from Koch's day. The question now is how far are we willing and able to go in our thinking and teaching.

It is clear by now that one element in the broader outlook I plead for is the inclusion of the environment in the consideration of causation of infectious disease, not as an unnatural concept but as a genuine consideration now supported, although incompletely, by significant controlled observations.

The environment in this connection is a gradient from the immediate surroundings of the parasite in the tissues of the host, to the distant conditions of region and climate so effectively emphasized by Tropical Medicine. I do not propose to enumerate all the stages in this gradient, and for purposes of simplicity I suggest that they be divided into two classes—the intimate and the remote.

Under the term "intimate" may be placed the cell and tissue constituents of the particular surroundings of the parasite, concerning whose various roles we know so little specifically. Why does a certain parasite flourish in a definite part of a tissue or organ of a given host species or strain of a single species? In general, we should consider that when an infection results from an agent that has circulated in the body, its successful localization is due to its finding a favorable intimate environment in a particular part. If I limit myself to considering the unimmunized animal, I should like to ask what is present or lacking in that certain site that favors or discourages microbic growth?

It is becoming increasingly evident that the age of the host is frequently significant, and evidence is accumulating that the nutritional state of the host plays a part in some instances. There are of course those more obvious factors that have to do with this matter of immediate environment, such as trauma and the route of entry of the agent into the animal host; even the numbers of the invading parasite may affect the local situation so that the few survivors of the new population assert themselves effectively.

We must recognize also that what we call a specific infectious disease is not always due to a single microbic species in pure culture. If the infection is of a mucous surface especially, other parasitic and ordinarily non-pathogenic forms may take a significant part in the process. This associative action of different agents sometimes takes a dramatic form, and in numerous instances there is a variation in the properties of the agents as well as a striking alteration of the infectious process itself. Here again, we must accept a modification of the former stereotyped concept of single specific causation of infectious disease and recognize that both etiologically and clinically the effect of the added microbes on the immediate environment of the initial parasite is significant. We err in thinking of the clinical entity of influenza as always being due solely to the influenza virus, of the common cold as being caused by the specific virus alone, of whooping cough as always attributable only to the pertussis bacillus, of smallpox as uncomplicated by bacterial etiology, and hog cholera as occurring naturally without added invasive bacteria.

These types of complex etiology are attributable in part to the physical opportunity of contaminating parasites to enter the lesion begun by specific

agents, but more pertinent to the argument is the thought that the intimate environment of a single parasitic species is modified by the associative action of other parasitic species.

The intensive study of viruses in recent years has only emphasized the importance of the role of host factors in the complex host-parasite relation. Virologists have been unhindered by artificial culture work and morphologic studies in their learning of viruses. They have perforce centered their attention on the environment of the virus as supplied by the living experimental host.

After referring to the cellular milieu of the parasite, so strikingly essential in the case of viruses, to the host factors of age and nutritional state among others, and to the associative action of different parasites in relation to etiology of specific disease, need I go further in illustrating the significance in medical Bacteriology of understanding the role of the intimate environment?

My next point needs no elaboration before this group. The remote environment, that is, remote from the parasite, has been amply demonstrated in Tropical Medicine, and during this war is being illustrated and recognized more and more. We think here of such factors as the temperature, altitude, humidity, local customs and living conditions, ectoparasites, and flying vectors of disease. Some of these factors are obvious in their effect, but some, particularly those that seem to exert an influence on the intimate host-parasite relationship, are not so well understood. But they are all factors of environment which we may call "remote" and which bacteriologists, virologists and other microbiologists must recognize above and beyond their laboratory knowledge of the specific agents themselves.

Where does all this bring us? To the proposition that new fields are available and open to Bacteriology and bacteriologists. After having explored its scientific region and established some of its basic principles and methods, Bacteriology is now prepared to turn to a closer study of Nature and to learn what the relation of the natural environment is to the various bacterial forms.

In connection with the free-living, non-parasitic types, the enlarged perspective means the consideration of ecology, in which studies are hardly begun and which in all its ramifications suggests a great variety of challenging, interesting and fruitful problems.

A careful consideration of the environment of

parasitic forms calls for a greater regard being paid to the animal in the host-parasite relation. Too often we have thought of the host as a test tube—inanimate, standard and originally sterile. But so common is the occurrence of a resident virus or bacterium in the experimental animal that virologists are obliged to be constantly on the guard in the study of an experimental virus. This illustrates the many less tangible but clearly natural factors that have to do with parasitism. The whole subject of the selective cellular milieu in the intimate environment of the parasite presents challenges unlimited in scope and promising in application.

Our information on the role of the environment remote from the parasite but affecting the host-parasite relation is as yet somewhat empirical and I fear we are in danger of allowing conceptions to be established that have no substantiation in scientific observation. This is a difficult problem for solution, this host-parasite-climate complex, but progress has been made and surely can be accelerated when the tools, ideas and vision of workers are brought to bear on it. I refer here not only to the environment of warm climates but also to the

larger problem of host-parasite-surroundings of all regions.

The war is the current event that is causing us to analyze our respective sciences in an attempt to see if we are progressing in keeping with our times and with the responsibilities we owe to society that nurtures science and currently fights for our liberty of study.

The review of my own science, Bacteriology, leads me to propose that its further development could profitably be in the direction of a higher level of perspective than perhaps now generally held. Without minimizing the importance of the study of bacteria under artificial conditions, the limitations of such methods should be recognized, and the significance of natural conditions and the environment is to be more fully appreciated. Natural conditions are complex and in reference to the parasite include both intimate and remote environmental factors. The importance of the environment is a principle of Tropical Medicine, illustrated and emphasized by world conditions of today. To the degree that Bacteriology, whether applied or pure, accepts this broader perspective, it will the better serve Natural Science and Society.



# DIARRHEAL DISEASES

## THE EIGHTH CHARLES FRANKLIN CRAIG LECTURE<sup>1</sup>

GEORGE R. CALLENDER<sup>2</sup>

*From the Army Medical School, Army Medical Center, Washington, D. C.*

### HISTORICAL

Diarrheal diseases are mentioned in the oldest documents of medical nature yet discovered. To quote from Wu lien-teh and co-authors in "Cholera," 1934 (1):

"In the Nei Ching—oldest and greatest Chinese classic on medicine attributed to the first Emperor Huang Ti (B.C. 2697-2597)—five main 'disturbances' of the human body were described, namely, of the extremities, heart, lungs, head and alimentary tract. To the last affection the two characters huo-luan were given, and have been handed down to the present day. The real meaning of huo-luan is sudden disturbance, which even in those early days was defined as a 'disturbance of the bowels and stomach.'"

No clear reference to diarrheal disease is found in the Egyptian papyri (2). In the Old Testament we find in the sanitary rules of the 23rd chapter of Deuteronomy the following:

"12. Thou shalt have a place also without the camp, whither thou shalt go forth abroad;

"13. And thou shalt have a paddle upon thy weapon; and it shall be, when thou wilt ease thyself abroad thou shalt dig therewith and shall turn back and cover that which cometh from thee."

It would be interesting to know the cause for such a regulation. Did it result from epidemic disease arising from the improper disposal of human excrement, or were the Children of Israel following the example set by the domestic cat? In the New Testament in the 28th chapter of Acts is recorded the sickness of the father of Publius, who rescued Paul at the time of his shipwreck on the Island of Malta. In the King James Version the condition is stated to have been "fever and a bloody flux," but in the Greek Testament the word "dysentery" replaces bloody flux, the latter still a term in use

in the British literature. The term dysentery appears more appropriate, for blood may be present without dysentery and dysentery be present without gross blood.

Earlier than the New Testament are the writings of Hippocrates in classic antiquity, approximately 460 B.C. (3). In these writings diarrhea and dysentery are differentiated. Hippocrates appeared to be aware that diarrhea leads to dysentery and that both increase in prevalence at the same periods. He recognized the ages and conditions in which mortality was greater and that cases in which flesh-like material was passed were serious while recognizable fever also indicated a more severe condition. In the "Definitiones Medicae," (4) about 450 A.D., attributed to Galen, a knowledge of the pathology of dysentery including phlegmon and ulceration is indicated. Early epidemics were described by Alexander Trallianus (5) in the Byzantine period, while Avicenna (6) in the Arabian period classified dysenteries by their supposed causes. The first recorded autopsy descriptions were by Benivieni in 1506 (7). It is interesting that ipecac was mentioned in Purchas' Pilgrimes in 1625 (8) and before this century was over had been used to a considerable extent in the treatment of dysentery especially in France.

The first organism considered as a possible cause of a diarrheal condition is described in a letter of Leeuwenhoek to Robert Hooke in 1681. This organism, as has been interpreted by Dobell (9) was the parasite now known as *Giardia lamblia*, described by Lambl in 1857 (10). In this same year Malmsten (11) discovered the large protozoan *Balantidium coli* in Scandinavia, and in 1875 Lösch (12) discovered the ameba of dysentery in man in Russia. It is interesting that these last two species, now more prevalent in tropical areas, were discovered in the temperate zone. In 1876 A. Normand (13) described the strongyloid which he found in cases of Cochín, China, diarrhea (*Strongyloides stercoralis*). In 1879 an important treatise on the subject of "alvine fluxes" was published in the medical history of the Civil War. This was written by Joseph Janvier Woodward (14) and

<sup>1</sup> Presented at the Thirty-Ninth Annual Meeting of the American Society of Tropical Medicine, Cincinnati Ohio, November 16-18, 1943.

<sup>2</sup> Colonel, Medical Corps, U. S. Army.

Note: Graph Number 2 is published by permission of War Medicine.



resulted from his studies of material collected at the Army Medical Museum from the battlefields of that war. His descriptions of the clinical course and postmortem pathology enable one to differentiate between amebic and bacillary dysentery and in some instances it has been possible to confirm the diagnosis by examination of the tissues still preserved in the Museum.

From 1883 to 1891 considerable advance in our knowledge of diarrheal disease was made by the Egyptian Cholera Commission (15). This commission differentiated endemic (amebic) and epidemic (bacterial) dysentery and demonstrated amebae in sections from the ulcerated intestine and in pus from liver abscess. About 1890 Councilman and Lafleur (16) introduced the term amebic dysentery. In 1897 Cassagrandi and Barbagallo (17) differentiated the harmless from the pathogenic amebae. Further work on this differentiation was done by Schaudinn about 1903 (18) although he left considerable confusion with reference to the pathogenic ameba, which was cleared up 10 years later by Walker and Sellards (19) in their work in experimental endamebic dysentery in man. The cultivation of the ameba by Boeck and Drbohlav (20) in 1925 was an important advance, which was followed in 1927 by the demonstration of the hemolytic, cytolytic, and complement binding properties of extracts of *Endameba histolytica* by Charles F. Craig (21).

The first bacterium proved to be a cause of bacillary dysentery is that now known as *Shigella dysenteriae*, discovered by Shiga and reported in 1897 (22). This was followed by the discovery of two organisms now classified as *Shigella paradyenteriae* in 1900, the first by Flexner (23) and the second by Strong (24). Since that time many other organisms classified in the *Shigella* group have been isolated under conditions which indicate that they are the cause of bacillary dysentery, while within the past 15 or 20 years it has become evident that many members of the *Salmonella* group are responsible for clinical dysentery even though the disease produced is ordinarily milder and more self-limited. The recent demonstration of common somatic antigens between the *Salmonella* and *Shigella* (25) is of considerable importance and indicates the possibility that many an etiologic bacterial factor of diarrheal disease has escaped recognition as such because of the impossibility of identifying it. The work of Edwards (26), Bornstein (27) and others in the serological and bio-

logical chemical differentiation of these groups and the improvements in media of the last decade make it possible to more nearly approach accurate knowledge as to the prevalence of inflammatory diarrheal disease.

From the days of Hippocrates to the beginning of this century the diagnosis of dysentery and the separation of simple diarrhea from the protozoan and bacterial lesions properly called dysentery was dependent upon clinical acumen. With the discovery and development of methods for the recognition of the parasites, clinical diagnosis went into the background in favor of the laboratory report and this happened before the laboratories were equipped to make the diagnosis in a significant proportion of the cases. The diagnosis of amebic dysentery is relatively easy, while even today with the most improved media a significant proportion of bacillary dysentery cases will not yield positive bacteriological results. There are, however, other microscopic methods which aid in the differentiation of the diarrheas.

In the last world war the diarrheal diseases, while apparently unimportant in United States troops, were responsible for serious non-effectiveness in the British forces especially in Mesopotamia. This high incidence of diarrheal disease, at first considered to be due to *Endameba histolytica*, was proved definitely to be bacillary in origin by the work of a number of men among whom Willmore and Shearman (28), Manson-Bahr, and Dobell played prominent parts (2). This study of the dysentery in the British Army showed the possibility of differentiating, first, diarrhea from dysentery by the presence or absence of an inflammatory exudate in the stool, and, second, the differential value of the character of the exudate in separating amebic and bacillary dysenteries. While the method does not have scientific laboratory accuracy, in the presence of large numbers of cases or in epidemics it can be used satisfactorily from the epidemiological standpoint and as an indication for the character of treatment. In the absence of laboratory facilities for the differentiation of the bacteria of dysentery, it can be utilized with a minimum of apparatus. It is especially valuable under field conditions and its utilization has been recommended under such conditions for troops in the present conflict.

The foregoing historical resume has to do with a group of diarrheal diseases of varied etiology. It does not consider the most fatal of all diarrheal

conditions—cholera. The authentic history of cholera is relatively brief. According to Wu lien-teh (1), it begins with the year 1817 when the disease broke out violently in Bengal. Undoubtedly it prevailed in India prior to that time but there is no definite evidence that it overstepped Asiatic limits prior to the spread to considerable parts of Asia and some parts of Africa in the years 1817 to 1823. Endemic foci exist in India especially in the delta of the Ganges and the river valleys of Assam and there are scattered areas where endemicity now exists far to the north along the China coast while occasional cases appear in the rural districts in the Philippines. Most of the epidemics appear to have spread from the endemic areas in India by land-traveled routes to Afghanistan, Persia, and southern Russia, and by sea along the coastal routes to Siam, Indo-China and the Chinese coast. America and Europe were involved in pandemics beginning in 1826, 1846, 1864 and 1892, while Europe has been visited even more frequently. This disease above all others in the diarrheal group indicates lack of sanitation particularly with reference to water supply. It is improbable that epidemics could occur with modern sanitary water supplies, and the disease cannot be considered a menace to this country. Its continued prevalence is entirely dependent upon the poor economic conditions which do not afford proper treatment of water, although local customs, especially some having a religious basis, have been anything but helpful in eradicating endemicity and frequently have been instrumental in starting epidemics.

*Vibrio cholerae* was discovered by Koch in 1883. These organisms have been studied extensively and today a number of types are described which appear to be of importance in disease production and in the manufacture of vaccines. There is still considerable work to be done in determining characteristics most desirable for immunization while the work with the vaccine has not been too well controlled. Sufficient evidence of the effectiveness of cholera vaccine exists, however, to cause its inclusion in the immunization program for troops operating in endemic areas. The destruction of the vibrios by the sera of man and animals immunized with the vaccine contributes to the confidence in its effectiveness. The organism is relatively easily lysed by bacteriophage although occasional strains are found during epidemics which are resistant, and specific phages for such strains have

been utilized. Experiments in which populations have been treated with phage have not been sufficiently controlled to render results of definite value. The treatment of drinking water with phage similarly appears to have given excellent results in certain instances but no adequate control groups as yet have been set up for this sort of prevention. The increased activity in study of the disease, however, caused by the present war may give rise to more definite knowledge of means of prevention.

The combatting of the dehydration of cholera by the use of saline solutions and water dates back one hundred or more years. Hypertonic saline solutions, introduced by Sir Leonard Rogers in about 1908 (29), have been effective in lowering the case fatality rate. Some reports of the use of the sulfonamide drugs have been favorable, others have not. Insufficient data are available to enable us to evaluate them. It is hoped that they will be used more extensively and in as early a stage of the disease as possible so that we may have an answer as to their effectiveness.

As is abundantly proven by British experience, soldiers living under field conditions may and do get cholera if they do not maintain the sanitary discipline necessary in endemic areas.

#### PROTOZOAN DYSENTERY

The prevalence of amebic infection is variously estimated from a low of some 5 percent in the adult population of temperate zones, to 50 percent or over in tropical and subtropical areas especially where economic conditions are poor. Actually it produces a relatively small part of diarrheal diseases. Its chronicity and secondary lesions outside of the intestinal tract, plus the occasional increases in incidence such as occurred in 1933 in Chicago (30), while tending to magnify its importance to some degree, have made the medical profession more alert in diagnosing the cases and in treating them intelligently. In many of the United States the disease is reportable and a considerable number of surveys have been made. An excellent summary of the available evidence on the prevalence of amebiasis in the Western Hemisphere was made by Faust in 1941 (31). I quote from his summary and conclusions: "Amebiasis surveys in the different geographical locations in the Western Hemisphere are too few and too fragmentary to provide a complete endemic picture of the infection. . . . The positive cases reported constitute only a fraction of the true incidence in the par-

ticular area." Verbal and unofficial reports from various areas considered to have high endemicity of amebic dysentery indicate that among allied fighting troops in these countries only 8 to 10 percent of the clinical dysenteries admitted to the hospital are caused by *Endameba histolytica*. Proportionately to bacillary dysentery, however, amebiasis leads to more chronic invalidism, especially if improperly treated, and does constitute a very definite problem not in battle line efficiency but in subsequent pensionable invalidism. Methods of treatment in use today offer a high probability of cure but are as yet not ideal.

Dysentery due to *Balantidium coli* is relatively infrequent and here again poor economic conditions are contributory. Man appears to be infected from the pig and contact with these animals appears always to be associated with reported cases. The organism is present in the pig practically universally. It is indeed rare to find a pig's intestine which at slaughter does not have an enormous number of these organisms in the intestinal content. Only rarely have cases of actual ulceration been found in these animals. The infection appears to have no significance from a military standpoint. The arsenicals stovarsol and carbarsone have been reported as effective in eradicating the parasite from man and recently there is a report of a successful treatment by diodoquine after failure with two of the arsenicals (32). It appears that man may harbor the parasite without any evidence of disease.

The status of other protozoan parasites, *Giardia lamblia*, *Trichomonas*, *Chilomastix* and other amebae with reference to their pathogenicity is not well defined. What evidence we have indicates that under certain conditions they may irritate that portion of the intestinal tract where they are commonly found and may thus lead to some activity on the part of bacteria normally present.

#### DIARRHEAL DISEASES DUE TO BACTERIA

##### a. *The shigella group*

This group of organisms, for years the only ones recognized as producing clinical dysentery, was established by the discovery of the dysentery and paradysentery groups. Especially during the last 20 years numerous organisms having serological and biochemical differences have been placed in the *Shigella* group and occasional new ones are discovered from time to time. Their prevalence is worldwide, certain types being distinctly more

prevalent than others. *Shigella dysenteriae* appears to vary from time to time in the same locality in the proportion of cases it produces. The paradysentery group likewise varies. *Shigella sonnei* of recent years has been quite prevalent in many geographical areas and it seems possible that this organism has been responsible for a considerable number of cases which would have been undiagnosed bacteriologically with ordinary methods of examination due to the atypical reactions on media of this species. A study of the bacteriological characteristics of organisms found in clinical dysentery by Boyd (33) has brought out a number of types which would not have been diagnosable with sera in use before his classification was made, and today in cultures especially from our soldiers abroad we are finding organisms which appear to belong in the *Shigella* group which we have not been able to differentiate with all the sera at our command. This condition might have been anticipated to some extent because of experiences with antidysenteric sera. The early sera produced were made by immunizing animals with some 31 strains. In Manila in 1922 a serum was being made containing 33 strains, 2 of which belonged in the *Salmonella* group, which was quite effective against all the dysentery cases I saw it used on in Manila. This same serum, however, was flown by airplane to the Island of Negros to treat children in an outbreak in a resident school. It appeared absolutely ineffective against the disease there. At that time serological differentiation of even the Flexner or paradysentery group was not possible. High endemicity for the group in general is again to be expected where economic conditions are poor, where privies breed flies or flies have access to them. The establishment of an Army camp, using field sanitary appliances, in areas of high endemicity is too often followed in the course of a few weeks by more or less diarrheal disease among the troops. In the last war this danger was practically eliminated in troops in the United States as shown by the rates in the official reports, because water disposal of sewage was established in all the cantonments and improvised or field latrines, always controlled with difficulty so far as fly ingress and breeding are concerned, were utilized to a very minor degree. In the maneuvers coincident with our mobilization for World War II, improvised field sanitation has been extensively utilized with an increasing and expected rise in diarrheal disease. Diarrheal disease prevention under field conditions

is dependent upon a very strict discipline and adequate methods of preventing contact between human feces and human food. The fly appears to be more of a menace, at least in this country, today with reference to dysentery than the water supply.

#### b. The *Salmonella* group

With the exception of the organisms causing the typhoidal fevers, this group has been associated largely with outbreaks of food infection or food poisoning. As a result of work during the last 20 years the importance of the *Salmonella* group in causing clinical dysentery has been brought out. The number of varieties of *Salmonella* is now large and scarcely a month goes by that someone does not find a new one. For the most part these organisms appear to be natural parasites of animals rather than man though man may carry them for various lengths of time. Not a few of them cause clinical dysentery which while usually self-limited may give rise to ulceration and lead to fatalities particularly in young children and the debilitated. A recent review by Bornstein (27) is an excellent presentation of the status of this group today. Aside from food poisoning, which may be sporadic or in epidemics more or less explosive in character, members of this group are usually found in any increase in diarrheal disease in a community and especially in such communities as troops under field conditions. Their presence simply indicates that they as well as the *Shigella* can infect man under similar conditions and produce clinical dysentery with the identical picture in the exudate as seen in the milder cases due to *Shigella* infection. In the food poisoning outbreaks likewise, provided infection occurs in addition to poisoning by the products of the bacteria, the stool exudate cannot be differentiated from that of *Shigella* infection. Clinically the enteritis is of the shorter duration and more self-limited in character, terminating without a long carrier state. Of recent years varieties found in chickens, ducks and other birds have come into prominence while *S. enteritidis*, ordinarily a parasite of cattle, has been found less frequently. *S. cholerae suis*, occasionally found especially in sausage poisoning outbreaks, is sometimes recovered from the blood and in not a few instances reported from the military service, has been confused with its antigenically identical cousin *S. hirschfeldii* or paratyphoid C. The actual prevalence, however, of *S. hirschfeldii* in this country appears to be low, and in fact the only area on which

I have any information of a considerable prevalence of this organism is in the Belgian Congo and Kenya (34). The general prevalence is so low that it has not been deemed necessary to include it in our typhoid vaccine. *S. typhi murium* often causes sporadic cases of the food poisoning or food infection type, for individual portions of food preserved as leftovers may be infected and the isolated or sporadic case result from contamination by mice or cockroaches.

Treatment of bacillary infections has included diarrhea mixtures of opium and bismuth, saline catharsis, serum and sulfonamide drugs. Serum has been spectacularly successful in some series especially when treatment is early, but it is manifestly impossible to include in the serum antibodies for all species. It also appears impractical to include in a vaccine for active immunization all the important antigenic factors in the groups of organisms responsible for bacillary dysentery. Sulfadiazine drugs have proven effective especially when given early. Convalescence is prolonged in proportion to the delay in instituting treatment. The sulfonamides stop the activity of the organism; time is required to heal ulcers.

#### STAPHYLOCOCCUS AND STREPTOCOCCUS

These organisms should be mentioned in connection with diarrheal disease. *Streptococcus* has been reported rarely as a cause of food poisoning but staphylococci, both aureus and albus, first described as agents causing food poisoning by Barber in 1913 (35), have assumed an important place in the causation of food poisoning.

Some but not all of the staphylococci found in food produce enterotoxic substances as shown by experiment in man and animals. Some will proliferate at icebox temperature and some will grow in agar media having the salt content of the brine used in pickling hams (10 per cent NaCl + 1 per cent KNO<sub>3</sub>) (36).

The symptoms of staphylococcus poisoning often differentiate it. Gastric pain, vomiting and collapse one to four hours after ingestion are rather characteristic. The vomitus may contain blood. Diarrhea may or may not follow. Exudate in the stools has not been described.

#### PREVALENCE OF DIARRHEAL DISEASES

It is practically impossible to make any definite statements as to the prevalence of diarrheal diseases. In few areas are dysenteries reported to

health services except as they are considered to be a cause of death. It is doubtful if more than 20 to 25 per cent of diarrheal diseases are ever brought to the attention of a physician. Only when they have lasted for a number of days or are of unusually severe character do they appear in any records. Civil records are of little or no value. A Public Health Service survey (37) of food poisoning and food infections in 1939, while admittedly incomplete, indicates that this one item in the diarrheal group is of very considerable importance in causing illness. The group as a whole is extremely important as a cause of infant mortality, especially in populations of a low economic status. Even in the military service, where there is every inducement to go early for treatment, many a case never appears on sick report especially under field conditions.

Throughout written history as relates to wars, diarrheal disease has been of great importance in producing non-effectiveness in troops. In many wars it has been the deciding factor in campaigns as it was in the last World War in the fighting in Mesopotamia. It is more important in wars fought in warm climates than in those in temperate climates although it may be serious anywhere. It was more important than any other one disease in our own Civil War (38). In the Spanish War there were more than twice as many cases of diarrheal disease admitted to hospital than there were typhoid, yet the deaths from the latter were nearly ten times those of diarrheal diseases. In World War I, as previously stated, diarrheal disease dropped to the lowest level in history, while typhoid fever had risen appreciably from the low attained following compulsory vaccination in 1911. Ordinarily we anticipate a simultaneous increase in diarrheal diseases and typhoid fever as both ordinarily present the same epidemiological picture. The reverse was true in World War I. Typhoid plus paratyphoid and dysentery both bacillary and amebic increased together in 1916 in the mobilization on the Mexican Border prior to this war. During this war typhoid rates were higher than those reached immediately following compulsory immunization but were only approximately a tenth of the rate preceding its use. From 1920 to 1939 both typhoid and the diarrheal disease rates, except for an epidemic of 22 cases of typhoid in 1931, remained at levels appreciably below those existing prior to 1916, and typhoid even with the mobilization of the present emergency has been so infrequent that the rates are insignificant (39).

Diarrheal disease on the other hand, reaching its lowest point in the history of our Army in 1919, maintained a very low level until about 1930, at approximately 12 to 14 per thousand per annum. In 1930 the rate increased to about 20 where it continued until 1939. In this year there was considerable activity of troops in the field increasing rapidly in 1940 and 1941, such increase being paralleled by the increase in the diarrheal disease rate until in 1941 it was practically 50 per thousand per annum, a rate at the level of 1905 and only exceeded from 1905 to 1941 by the sharp rise of the 1916 epidemic on the border. Figure 1 shows both typhoid and diarrheal disease for the period starting with 1900. Included in the diarrheal disease group are the diagnoses grouped together under the same basic number in the reports of the Office of the Surgeon General. These include diarrhea cause not specified, acute gastritis and enteritis, colitis and other enteritis, and dysentery. The typhoid rates include paratyphoid beginning with the year 1912 but except for the sharp rise of 1916 and throughout the first World War, paratyphoid has not influenced rates significantly.

Figure 2 illustrates the admission rates for diarrheal diseases from 1900 to 1941 and the specific diagnoses, dysentery, amebic, and dysentery, bacillary. The second line from the top represents diarrhea and enteritis while the specifically diagnosed diarrheal diseases are represented by the narrow space between the two upper lines. It appears obvious that only an inappreciable number of diarrheal diseases are specifically diagnosed.

Specific diagnoses were first shown in the Surgeon General's Report in 1905. It will be noted that up to and including 1916 the diagnosis of amebic disease follows the trend of the main curve while the bacillary curve bears no relation to it. The sharp jump in amebic disease in 1920 was due to erroneous diagnosis, an enlisted technician's word being accepted for the presence of *Endameba histolytica* when in fact he was so diagnosing macrophages containing red corpuscles. The sharp drop in bacillary dysentery should have been a rise, and amebic disease should have shown no fluctuation from its ordinary level. As a result of the publication in 1922-24 (40, 41, 42) of papers emphasizing the happenings of the immediately preceding years, especially in publications reaching medical officers, reported incidence of bacillary dysentery for the first time rose above that of amebic infection. The peaks in the bacillary

group in 1927, 1930 and 1935 were each caused by epidemics in troops in the field under field sanitary conditions. It will be noted that diagnosis appeared to be slipping again about 1935 and even in 1941 an insignificant number of positive specific diagnoses were being recorded.

It does not appear that such lack of proper diagnosis is necessary. The determination of whether

bers of cases are involved, positive bacteriological diagnosis of a few to indicate the probable type involved is all that is necessary, and in advanced stations away from suitable laboratories the diagnosis of dysentery can be made without bacteriological diagnosis as it was throughout all the years prior to the discovery of the organisms. I have not mentioned the clinical diagnosis without the

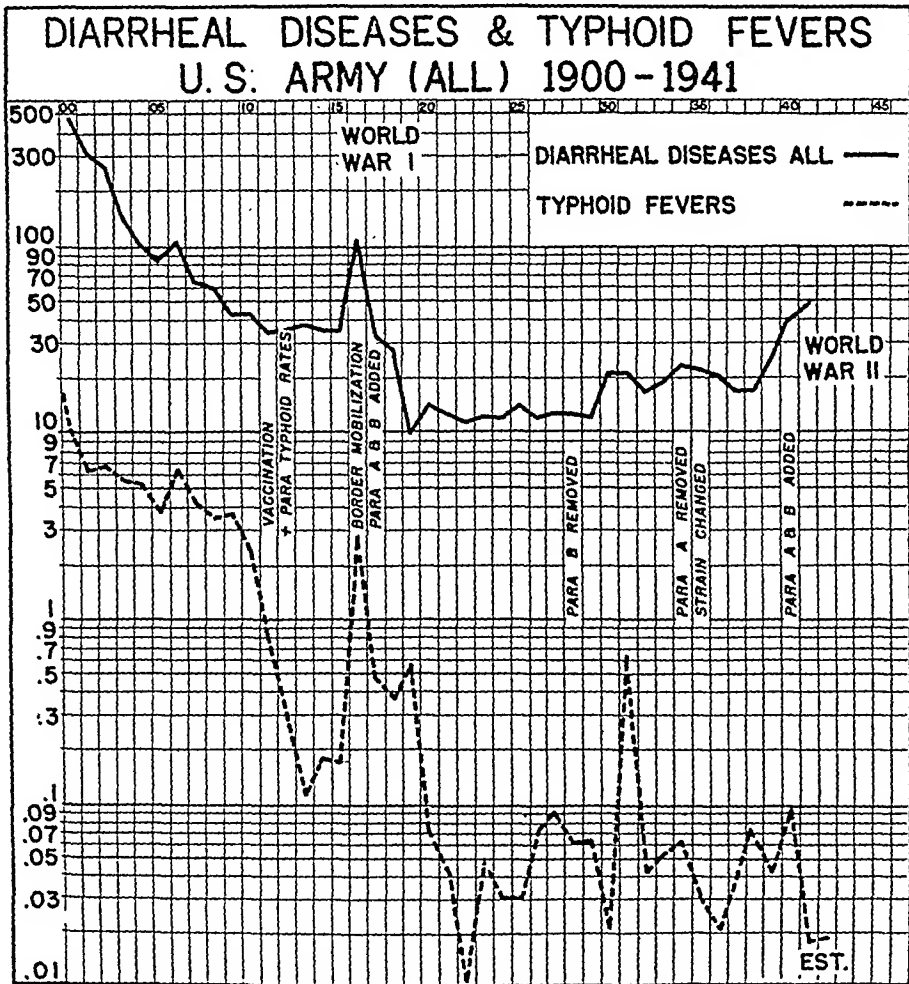


FIG. 1

an inflammatory disease of the colon exists is a matter requiring but a few minutes. An applicator stick or a loop to obtain material from the stool, a glass slide and a little dye, and an examination lasting one or two minutes under the microscope will differentiate inflammatory from non-inflammatory lesions. Treatment can be instituted at once and proper measures taken to prevent further spread of the condition. Where considerable num-

bers of cases are involved, positive bacteriological diagnosis of a few to indicate the probable type involved is all that is necessary, and in advanced stations away from suitable laboratories the diagnosis of dysentery can be made without bacteriological diagnosis as it was throughout all the years prior to the discovery of the organisms. I have not mentioned the clinical diagnosis without the

spite of the presence of microscopic pus and leukocytes in the stool, the clinicians were reluctant to diagnose it as bacillary dysentery because there was no pus or blood visible to the naked eye.

It is earnestly recommended that in the study of diarrheal disease and in its diagnosis, whatever may be the indication, that the stool should be examined (1) for the presence of exudate, (2) for the character

proper position as no important part of diarrheal disease incidence.

#### SUMMARY AND CONCLUSIONS

1. In the past 40 years while it has been possible to differentiate the various causes of diarrheal disease, this has not been done. As a result available data indicate their prevalence only when all diagno-

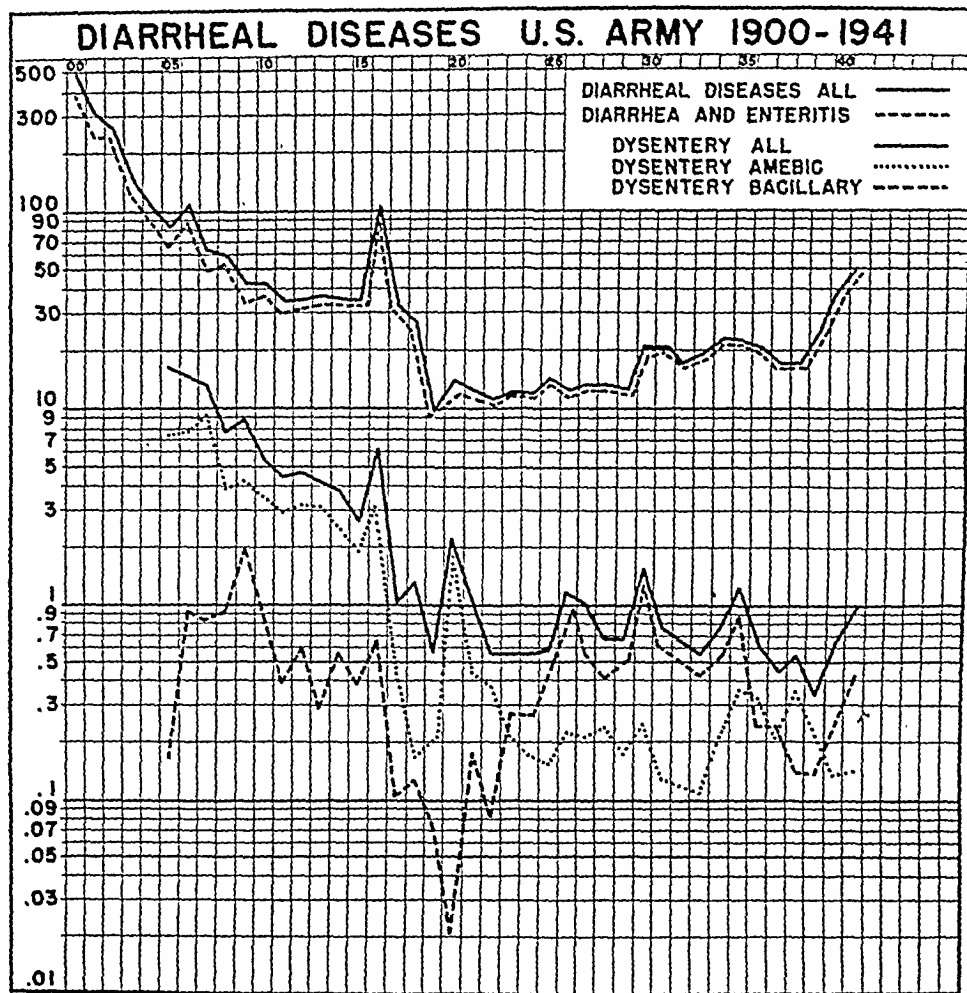


FIG. 2

of the exudate, (3) for the presence of protozoan or other parasites, and (4) bacteriologically for the causative organism. The doing of any one of this group without the others leads to a false picture of the condition present and has made our statistical data essentially worthless. If this program were followed, I believe that the diagnosed cases would assume their appropriate position on the chart and that common diarrhea would also assume its

ses, diarrhea, dysentery, gastroenteritis and enterocolitis, are grouped under one heading.

2. An inappreciable proportion of actual bacillary dysentery is so diagnosed but amebic dysentery is diagnosed in a much larger proportion of the total cases.

3. The use of improvised sanitary installations for the disposal of human waste by troops requires an excellence of sanitary discipline difficult to



attain, while the use of unsanitary installations for the same purpose in civil life produces a seedbed which maintains high endemicity and forms the opportunity for epidemics to occur.

4. Typhoid and dysentery ordinarily increase under the same unsanitary conditions as is proven by past experience in armies. In the present war typhoid vaccine appears to have controlled the incidence of this disease, while bacillary dysentery for which no immunization has been developed has risen in troops to heights equal to those of 20 years ago.

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# MENINGITIS ON THE ISTHMUS OF PANAMA<sup>1</sup>

B. H. KEAN<sup>2</sup> AND W. D. CRANDALL<sup>3</sup>

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## INTRODUCTION

Our interest in this problem stemmed from two main sources:

(1) The geography of disease is coming to be an increasingly important subject and it was felt that a comparison of meningitis on the Isthmus of Panama with meningitis elsewhere might be valuable.

(2) The race<sup>4</sup> incidence of disease in Panama is worthy of study. It has already been established (1, 2) that the incidence of hypertension is much higher in West Indians living on the Isthmus of Panama than in Panamanians. It was not known whether there were racial differences in other diseases, such as meningitis.

Certain definitions have been employed in this paper. By *bacterial meningitis* is meant, broadly speaking, purulent meningitis, characterized by an increased number of neutrophilic leukocytes in the spinal fluid and the presence of subarachnoid exudate. It includes meningococcus, pneumococcus, streptococcus, staphylococcus, *Hemophilus influenzae* meningitis, and, by definition in this paper, tubercle bacillus meningitis. It does not include lymphocytic choriomeningitis, syphilitic meningitis, or hemorrhagic pachymeningitis.

By *Panamanian* is meant a Latin American, born in Panama, both of whose parents were born in Panama. By a *West Indian* is meant a Negro born in the West Indies of Negro parents, or a Negro born in Panama, both of whose parents were born in the West Indies. During the construction period of the Panama Canal thousands of negroes were imported from the West Indian Islands, especially from Jamaica and Barbados, and it is to these

negroes and their descendants that the term West Indian is applied.

The data for this study were obtained from the case records of Gorgas Hospital, Ancon, Canal Zone (known until 1927 as Ancon Hospital). All cases of purulent meningitis from May, 1904, when the hospital was taken over by the United States authorities, to January 1, 1942, were included. The autopsy reports were taken from the records of the Board of Health Laboratory, Gorgas Hospital.

During the period surveyed there were 457,701 hospital admissions (see table 1). In 524 instances the final diagnosis was purulent meningitis; the average incidence of purulent meningitis, therefore, was 1.14 per 1,000 hospitals admissions. The average number of hospital admissions per year was 12,044 and the average number of cases of meningitis was 13.8. The number of cases of meningitis per year varied from 1 in 1904 to 34 in 1927. Except for meningococcus meningitis, no significant annual variations were noted. Of the 524 cases, 455 or 86.8 per cent died. Complete autopsies, including examination of the head, were performed on 346 patients or 66 per cent of the total group and 76 per cent of those who died.

In all our statistical studies two parallel charts were constructed: (1) upon the entire group of 524 cases in which the final diagnoses were made by the clinicians, based, of course, almost exclusively upon the bacteriologic findings, and (2) upon the autopsy series of 346 cases in which the diagnosis was made by the pathologist. No significant differences were found, in any respect, between these two series. For this reason we regard the clinical diagnoses on the 180 patients upon whom autopsies were not performed as thoroughly reliable and therefore have presented the data on the total of 524 cases.

## THE DATA

### *Types of meningitis*

The types of meningitis and their percentages are recorded in table 2 and compared with statistics from New York, Chicago, and New Orleans. The New York statistics were compiled by Neal (3) and

<sup>1</sup> From the Board of Health Laboratory, Gorgas Hospital, Ancon, Canal Zone.

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<sup>2</sup> Lieutenant, Medical Corps, Army of the United States (Pathologist).

<sup>3</sup> Lieutenant, Medical Corps, United States Naval Reserve.

<sup>4</sup> In this paper the term race is used colloquially, meaning a group of people with similar appearance, background, customs, and language.

represent cases which "occurred in or near New York City between the years 1910 and 1940" (4). The New Orleans series was reported by Tripoli (5)

pital from January 1, 1937 to February 1, 1940; this series includes 18 cases of lymphocytic choriomeningitis listed in Table II under "all others."

TABLE 1  
*Incidence of bacterial meningitis 1904-1941 among admissions to Gorgas Hospital*

YEAR	GORGAS HOSP. AD- MISSIONS	TUBER- CLE BACILLUS	PNEUMO- COCCUS	MENINGOCOCCUS*	STREPTO- COCCUS	STAPHY- LOCOCCUS	H. INFLU- ENZAE	ALL OTHERS	TOTAL	NO. PER 1,000 AD- MISSIONS
1904	1,173	1							1	.85
1905	7,570		2		2				4	.53
1906	13,218		13	2	2			2	19	1.44
1907	14,237		5	8(1)				3	16	1.12
1908	15,880		2				1		3	.19
1909	18,960	2	1	2					5	.26
1910	20,491	4	2	1	2			2	11	.54
1911	15,344	2	3					1	6	.39
1912	14,494	2	10	3	2	1			18	1.24
1913	15,280	2		4(2)				3	9	.59
1914	15,425	3	4	1				1	9	.58
1915	10,652	6	2	3(1)	2				13	1.22
1916	9,116	3	3	1(1)				1	8	.88
1917	10,880	3	1	5(1)				1	10	.92
1918	12,153	9	4	9(7)	4			1	27	2.23
1919	10,503	7	2	4(1)	4			1	18	1.72
1920	9,783	5	3	4	1	2		1	16	1.64
1921	8,146	4	3	1(1)	1				9	1.10
1922	6,243	2	1			1	1	3	8	1.28
1923	6,343		1	1	1		1	4	8	1.26
1924	7,451	4	1		1			3	9	1.20
1925	8,125	4	2	1(1)	3				10	1.23
1926	7,525	5	2	4(2)				1	12	1.60
1927	9,026	6	4	19(11)	2	1		2	34	3.76
1928	12,089	5	4	7(2)	1	1		3	21	1.74
1929	11,535	8	2	10(7)	1	1	1		23	2.00
1930	12,005	4	1	4	1			2	12	1.00
1931	12,239	7		6(1)		1		2	16	1.31
1932	11,330	10	7	1(1)	3	1		1	23	2.04
1933	11,621	7	3	1(1)				1	12	1.03
1934	9,630	3	8		1			5	17	1.77
1935	10,692	7	4	1	1	1	1	4	19	1.78
1936	12,131	4	3	4(2)		2	1	4	18	1.48
1937	13,217	3		2(1)		1	1	4	11	.83
1938	12,679	8	6	4			3	1	22	1.74
1939	12,060	7	3		2		1	2	15	1.24
1940	20,964	5	4	2(1)	1		3		15	.72
1941	27,491	5	4	4		2		2	17	.62
Total.....	457,701	157	120	119(45)	38	15	14	61	524	1.14
Yr. av.....	12,045								13.8	

\* Figures in parentheses indicate patients with meningococcus meningitis who were transients.

from the Charity Hospital in New Orleans and covers the ten year period between 1925 and 1934. The Chicago series was collected by Rhoads and others (6) from admissions to Cook County Hos-

#### *Racial incidence of meningitis*

Data are provided in tables 3 and 4. From table 3 it will be seen that of all patients upon whom autopsies were performed at the Board of Health

Laboratory, there were five times as many West Indians as Panamanians and six times as many West Indians as United States citizens. Nevertheless, there were only two to two and one-half times as many West Indians as Panamanians with meningitis. Table 4 reveals that there were only twice as many West Indians with tubercle bacillus and pneumococcus meningitis as Panamanians.

both sexes were found, but could be explained by the sex distribution of the population. For example, among the United States citizens with meningitis the ratio was 11 males to each female, an expression of the presence of a large soldier population and the fact that many patients came from army transports. In the native population the ratio of males to females was 2:1.

TABLE 2  
*Incidence of types of bacterial meningitis*

TYPE OF MENINGITIS	ISTHMUS OF PANAMA, GORGAS HOSPITAL		NEW YORK		CHICAGO, COOK COUNTY HOSPITAL		NEW ORLEANS, CHARITY HOSPITAL	
	Number of cases	Per cent of total	Number of cases	Per cent of total	Number of cases	Per cent of cases	Number of cases	Per cent of total
Tubercle bacillus.....	157	29.9	1045	27.6	158	34.4	51	10.9
Pneumococcus.....	120	22.9	316	8.4	71	15.5	111	23.7
Meningococcus.....	119 (45)*	22.7	1638	43.3	105	22.9	221	57.2
Streptococcus.....	38	7.2	323	8.5	42	9.1	24	5.1
Staphylococcus.....	15	2.9	43	1.2	3	0.7	9	1.9
<i>H. influenzae</i> .....	14	2.7	197	5.2	29	6.3	20	4.3
All others.....	61	11.7	218	5.8	51	11.1	32	6.9
	524	100	3780	100	459	100	468	100

\* Number of transients.

TABLE 3  
*Race distribution*

	CASES OF MENINGITIS (TOTAL GROUP OF 524 CASES)		CASES OF MENINGITIS (AUTOPSY GROUP OF 346 CASES)		TOTAL AUTOPSIES DONE AT BOARD OF HEALTH LABORATORY 1904-1942*	
	Number	Per cent	Number	Per cent	Number	Per cent
West Indians.....	227	43.3	181	52.3	7,606	64.6
Panamanians.....	97	18.5	60	17.3	1,442	12.3
U. S. A.....	88	16.8	39	11.3	1,236	10.5
Other Groups.....	112	21.4	66	19.1	1,488	12.6
Total.....	524	100	346	100	11,772	100

\* Note: During the period 1904-1942 a total of 13,096 autopsies were done. If stillbirths and those cases in which information concerning race are excluded, 11,772 autopsies remain.

Among the patients with meningoboccus meningitis the ratio of West Indians to Panamanians was 5:1, the expected ratio. A striking finding was the presence of four times as much *H. influenzae* meningitis in the very much smaller group of Panamanians as in the larger group of West Indians.

#### Sex

Seventy per cent of the patients were males, but approximately 75 per cent of the hospital population has been male. Significant differences in the distribution of the various types of meningitis in

#### Age

In tables 5 and 6 are recorded the age and race distribution of the patients with the various types of meningitis. Most of the Panamanians with meningitis were under 5 years. The age distribution among West Indians was more generalized. Most of the United States citizens were young soldiers between 18 and 25 years. Fifty per cent of the patients with meningococcus meningitis were between the ages of 16 and 25 years. Among the patients with tubercle bacillus meningitis 60 per

cent were 5 years of age or less. Of the 14 patients with *H. influenzae* meningitis 9 were under the age of 2 years.

#### Death rates

All patients with tubercle bacillus, pneumococcus, and staphylococcus meningitis died. Ninety-

five per cent of those with streptococcus meningitis died (2 patients in 38 survived). Eighty-five per cent of those with *H. influenzae* meningitis died (2 patients out of 13 recovered). The sulfonamides were not used in treating the patients with streptococcus meningitis and *H. influenzae* meningitis who recovered. Of the patients with meningococcus

TABLE 4  
Race incidence of meningitis\*

SPECIFIC TYPE OF MENINGITIS	TOTAL NUMBER OF CASES	WEST INDIANS (CASES OF MENINGITIS—227)			PANAMANIAN (CASES OF MENINGITIS—97)			UNITED STATES (CASES OF MENINGITIS—88)		
		No. of cases of specific type of meningitis	Per cent of West Indians	Per cent of specific type of meningitis	No. of cases of specific type of meningitis	Per cent of Panamanians	Per cent of specific type of meningitis	No. of cases of specific type of meningitis	Per cent of U. S. citizens	Per cent of specific type of meningitis
Tubercle bacillus...	157	72	31.7 (72÷227)	45.9 (72÷157)	38	39.2 (38÷97)	24.2 (38÷157)	6	6.8 (6÷88)	3.8 (6÷157)
Pneumococcus.....	120	60	26.4 (60÷227)	50.0 (60÷120)	31	32.0 (31÷97)	25.8 (31÷120)	7	7.9 (7÷88)	5.8 (7÷120)
Meningococcus.....	119	29	12.8 (29÷227)	24.4 (29÷119)	6	6.2 (6÷97)	5.0 (6÷119)	58	66.0 (58÷88)	48.7 (58÷119)
Streptococcus.....	38	24	10.6 (24÷227)	63.1 (24÷38)	4	4.1 (4÷97)	10.5 (4÷38)	2	2.3 (2÷88)	5.3 (2÷38)
Staphylococcus.....	15	6	2.6 (6÷227)	40.0 (6÷15)	1	1.0 (1÷97)	6.7 (1÷15)	5	5.7 (5÷88)	33.3 (5÷15)
<i>H. influenzae</i> .....	14	2	0.9 (2÷227)	14.3 (2÷14)	8	8.2 (8÷97)	57.1 (8÷14)	1	1.1 (1÷88)	7.1 (1÷14)
All others.....	61	34	15.0 (34÷227)	55.6 (34÷61)	9	9.3 (9÷97)	14.8 (9÷61)	9	10.2 (9÷88)	14.8 (9÷61)
Total.....	524	227	100		97	100		88	100	

\* In this table, data on "Other Groups," meaning patients of mixed "race," various Latin American peoples and representatives of a score of countries, have been united for purposes of simplification.

TABLE 5  
Bacterial meningitis: distribution by racial groups and age

AGE	PANAMANIAN	WEST INDIANS	U. S. A.	OTHER GROUPS	TOTAL
0-5	62	74	6	47	189
6-10	8	9	3	11	31
11-15	2	6	3	8	19
16-20	4	14	25	4	47
21-25	6	28	29	13	76
26-30	5	21	7	8	41
31-40	7	29	6	6	48
41-50	0	20	4	3	27
Over 50	1	18	3	1	23
Not stated	2	8	2	11	23
Total.....	97	227	88	112	524

meningitis 52 per cent died. The death rate for the United States citizens with meningococcus meningitis was 43 per cent and for West Indians, 80 per cent.

#### DISCUSSION

##### General comment

A consideration of the foregoing data suggests that bacterial meningitis is not rare in the tropics if the Isthmus of Panama can be used as a criterion. The high mortality of this group of diseases makes meningitis as serious a problem on the Isthmus of Panama as in some urban centers of the United States. The types of meningitis seen on the Isthmus of Panama are essentially similar to those in the United States, but the proportions vary considerably.

*Tubercle bacillus meningitis*

Tuberculosis ranks first as a cause of death in the Republic of Panama (7). The high incidence of tubercle bacillus meningitis reflects the high general incidence of tuberculosis. As more attention is paid to the problem of tuberculosis in the Republic of Panama, a decrease in this type of meningitis may be anticipated.

*Pneumococcus meningitis*

It will be observed (table 2) that the relative incidence of pneumococcus meningitis is much higher on the Isthmus of Panama than in New York or Chicago, but the same as that in New Orleans. In 207 consecutive autopsies performed at the United Fruit Company Hospital at Tela,

cago. Further study of the geography of pneumococcus meningitis might be enlightening.

Typing of the pneumococci was accomplished in 41 instances. The following types were recorded: Type I, 6 cases; Type II, 6 cases; Type III, 9 cases; Type X, 1 case; Type XII, 2 cases; Type XXIII, 1 case; Type XXIV, 1 case. Fifteen cases were included in the old Type IV.

*Meningococcus meningitis*

The relative incidence of meningococcus meningitis on the Isthmus of Panama is half that of New York and New Orleans. This fact is especially striking if we realize that of the 119 cases included in tables 1 and 2, 45, or 38 per cent, were transients (indicated by parentheses in table 1). By "tran-

TABLE 6  
*Bacterial meningitis: distribution by type and age*

AGE	TUBERCLE BACILLUS	PNEUMO- COCCUS	MENINGO- COCCUS	STREPTO- COCCUS	STAPHYLO- COCCUS	H. INFLU- ENZAE	ALL OTHERS	TOTAL
0-5	92	38	18	7	3	10	21	189
6-10	19	1	5	2	0	1	3	31
11-15	4	3	5	1	2		4	19
16-20	7	7	27	1	1		4	47
21-25	11	14	34	6	2		9	76
26-30	12	12	8	3	1	1	4	41
31-40	9	15	6	11	2	1	4	48
41-50	2	10	1	4	4		6	27
Over 50	0	11	3	2	0	1	6	23
Not stated	1	9	12	1	0		0	23
Total.....	157	120	119	38	15	14	61	524

Honduras, from November 1922 to December 1925, Clark (8) encountered 32 or 15.4 per cent patients with meningitis. There were 27 cases of pneumococcus meningitis, 2 cases of tubercle bacillus meningitis, and one each of streptococcus, staphylococcus, and mixed meningitis. The large number of patients with lobar pneumonia treated at that hospital was responsible for the high incidence of this type of meningitis. A similar situation prevailed on the Isthmus of Panama during 1906 and 1912 (see table 1).

The morbidity and mortality from the pneumococcal diseases are generally considered to be highest in northern or temperate climates featured by inclement weather. Yet the relative incidence of pneumococcus meningitis is higher in New Orleans, Panama, and Honduras than in New York or Chi-

sients" we mean those who developed meningitis before arrival on the Isthmus or within ten days after arrival. Most of these were soldiers from transports. The high rate in 1927 is a reflection of the epidemics of meningococcus meningitis in army camps in the United States in 1927 (9). The annual incidence suggests (see table 1), however, that this disease may be endemic in Panama. Contact with transients may play a more important role than is recognized, and it is interesting to speculate whether meningococcus meningitis would survive on the Isthmus if outside contacts ceased.

*Streptococcus meningitis*

The uniformity of the percentages of streptococcus meningitis in the various localities (table 2) is worthy of note. It is the impression of the

senior author that the streptococcus diseases, or the diseases considered by many as associated with the streptococcus, e.g., puerperal sepsis, septic sore throat, scarlet fever, and rheumatic fever, have a lower morbidity and a lower mortality on the Isthmus of Panama than in the United States. This impression is not confirmed in regard to streptococcus meningitis, at least.

#### *Hemophilus influenzae meningitis*

The isolation of the responsible organism of this type of meningitis has been more successful in recent years than previously. Fothergill (10) has pointed out that during the period from 1933 to 1936 *H. influenzae* meningitis was the type most frequently seen in the Children's Hospital in Boston. Although the series is small, the presence of 8 of our 14 cases of *H. influenzae* meningitis in the smaller group of Panamanians, as compared to 2 cases in the much larger group of West Indians, is noteworthy, but no explanation is forthcoming. On the Isthmus of Panama, as elsewhere, *H. influenzae* meningitis is primarily a disease of young children, generally under the age of 2 years.

#### *Race incidence*

The variations in the race incidence of meningitis must be interpreted in the light of the unusual population groups seen at Gorgas Hospital. The United States citizens are composed essentially of two main groups, 1) "Gold" (white) employees of The Panama Canal and their families, 2) soldiers from the many army posts. The health of both groups is excellent and not comparable with that of the general civilian population in the United States. The only type of meningitis which is at all prevalent is meningococcus meningitis. It is to be expected that the incidence of the other types of meningitis would be low in this "race" group.

The West Indian and Panamanian populations resemble, to a much greater extent, general populations elsewhere and may be compared with each other. It will be observed in table 3 that in the autopsy population at the Board of Health Laboratory there are five times as many West Indians as Panamanians. We feel that this figure truly represents the ratio of the two race groups as seen at Gorgas Hospital. Nevertheless, there are only two to two and one-half times as much meningitis among the West Indians as among the Panamanians. While complete reliance upon the statistics is not justifiable, the impression that there is more

meningitis among Panamanians than West Indians is inescapable. As will be noted in table 5, the presence of much meningitis in Panamanian children under the age of 5 years is responsible to a great extent for the high incidence in Panamanians. Whether "racial susceptibility," poorer housing conditions, poorer nutritional states, or other factors are responsible, is not clear. The predominance of *H. influenzae* meningitis in Panamanians has already been mentioned.

#### SUMMARY

1. In 457,701 admissions to Gorgas Hospital from May 1904 to December 31, 1941, there were 524 cases of bacterial meningitis, the incidence being 1.14 per 1,000 admissions.

2. Of the 524 cases, 157 or 29.9 per cent were caused by the tubercle bacillus, 120 or 22.9 per cent by the pneumococcus, 119 or 22.7 per cent by the meningococcus, 38 or 7.2 per cent by the streptococcus, 15 or 2.9 per cent by the staphylococcus, 14 or 2.7 per cent by *H. influenzae*, and 61 or 11.7 per cent by other or mixed organisms.

3. The relative incidence of pneumococcus meningitis on the Isthmus of Panama is higher than that in series from New York and Chicago, but the same as in New Orleans. The relative incidence of meningococcus meningitis on the Isthmus of Panama is half of that in series reported from New York and New Orleans; the incidence is somewhat less than in Chicago if transients are excluded from the Panama series.

4. The impression is gathered that Panamanians are more susceptible to meningitis than West Indians. The incidence of meningitis in Panamanian children, especially *H. influenzae* meningitis, is remarkably high.

5. The death rate for all cases of meningitis was 86.8 per cent. All patients with tubercle bacillus, pneumococcus, and staphylococcus meningitis died. Two out of 38 patients (5 per cent) with streptococcus meningitis, and 2 of 13 (15 per cent) with *H. influenzae* meningitis recovered. Half of the patients with meningococcus meningitis died. The death rate of patients with meningococcus meningitis was about twice as high in West Indians as in whites.

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# OBSERVATIONS ON THE RELATIVE ATTRACTIVENESS OF MAN AND HORSE FOR *ANOPHELES ALBIMANUS* WEIDEMAN<sup>1</sup>

ALBERT A. WEATHERSBEE<sup>2</sup>

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The preferential blood supply of mosquitoes has been the subject of much speculation because of the direct relation it bears to the biological ability of a species to act as a vector of a disease. Observations on the blood feeding habits of mosquitoes have been recorded by a number of persons. Le Prince and Orenstein (1), (1916) observed that in Panama horses were more attractive to *Anopheles albimanus* than were men. Earle and Howard (2), (1936) found that this species in Puerto Rico exhibited a marked preference for horses and oxen over man, with pigs and goats about equalling man in attractiveness.

The determination of blood meal by the ring precipitin test as advanced by King and Bull (3), enables observers to determine with a high degree of accuracy the source of blood of engorged females, which reflects the preferential feeding habits of a species. They were also able to show that a relatively small percentage of female *Anopheles quadrimaculatus* feeding on man could maintain malaria at a high level in a community. By utilizing this method Davis and Shannon (1928) (4), were able to demonstrate that in Northern Argentina where *Anopheles pseudopunctipennis* is a serious vector of malaria, the frequency with which the species fed on man as compared with other animals was as high as 50 per cent. In Central America and in North America this species is of little importance as a vector of malaria, possibly because of differences in its selection of its blood hosts. Other species of mosquitoes elsewhere are known to be important vectors in some localities and unimportant in others. This situation led to the realization of the existence of races or biological varieties within certain groups of mosquitoes that had previously been regarded as being one species (5). The extent of such variations has not been completely determined, and the premise that variations not yet recognized may account for corresponding

variations in malaria transmission by an accepted species can not be disregarded.

In malaria control in Eastern Puerto Rico it was desired to determine the relative attractiveness of man and of horse for *Anopheles albimanus* for several reasons. The observations by Earle and Howard (2), were not made primarily in the particular area with which this report deals. The South shore of the island where many of their observations were made represents climatic and meteorological conditions considerably different from those in the area concerned. It was considered that observations particularly applicable to the area were desirable. Because of the difficulty of securing recently engorged female *Anopheles albimanus* in nature, the ring precipitin test could not be used as an indicator of the blood feeding habits of this species in this area. Moreover, it was also desired to use standardized human bait collections for determining periodically the *Anopheles albimanus* density within areas under control for comparison with the same in uncontrolled areas. In some countries this method is convenient and reliable (6, 7). However, before it could be practiced as a means of collecting *Anopheles albimanus* it was necessary to ascertain its reliability. These observations were therefore undertaken.

## PROCEDURE

A land-lock fresh water pond was discovered to be producing *Anopheles albimanus* abundantly. Two Magoon type animal bait traps (8), were located 100 feet apart about 200 feet from the pond, in such manner that neither would occupy a more favorable position than the other. In one of the traps a cot with bedding and mosquito netting was fitted so that a man might occupy the trap overnight without being exposed to the bites of mosquitoes entering the trap. For twenty nights this trap was occupied by a man. The other trap was occupied by a horse at the same time. Ten different men and six different horses were used in the observations. Each man occupied the cage trap at least two nights in succession, and each horse

<sup>1</sup> The expressions contained in this paper are the private ones of the author and are not intended to represent official views of the Navy Department.

<sup>2</sup> Lieutenant Commander, U.S.N.R.

occupied the horse trap two nights, but changes of men and of horses were made on alternate nights so that the attraction offered by a horse could be compared with that offered by not only one man but two or more as a means of equalizing individual variations in attractiveness of any particular man or horse. By this arrangement the attractiveness of three horses each could be compared with that of four different men, and three other horses with two different men. Six of the men engaged were white, three of which were continentals, two were mulattoes and two black. Horses of different colors were also used.

The man and the horse selected to occupy the traps entered simultaneously at 5:00 P.M. Each cage was then locked until 7:00 A.M. of the following morning, when both were released from their respective traps. Mosquitoes present inside of the traps were collected immediately and brought to the laboratory for identification. Observations were begun on March 1, 1943, and terminated on the morning of March 31, 1943, though no observations were made on seven of the intervening nights.

On the last two nights of the experiment, an attempt was made to determine the comparative attractiveness of horse and cow. However, the area in which the experiment was being conducted had been populated by personnel to whom it was desired to give as much protection against malaria as possible. A channel connecting the pond with the sea was therefore constructed and the salinity of the pond water increased from 15 per cent to 100 per cent of sea water concentration. After this time production of *albimanus* in the pond ceased completely. Collections of adults after increasing the salinity of the water declined rapidly until at the time of the experiment with the cow the numbers were too few on which to base comparisons. However, cows are employed satisfactorily as bait elsewhere in Puerto Rico.

#### RESULTS

Collections of *Anopheles albimanus* made in this experiment are summarized in table 1, from which it will be seen that throughout the experiment a total of 123 *albimanus* were collected after entry into the trap occupied by man and 2791 in the trap occupied by horse during the same time. This represents a ratio of 1 to 22 in favor of the horse. The maximum number entering the man-trap per night was 19, as compared with 364 for the horse trap; a ratio of 1 to 19. The greatest difference in

attractiveness observed occurred on the night of March 2, or in the collections made the morning of March 3, when the ratio was 1 for man to 285 for horse. In considering the mean number per catch, 6.1 for man and 126.9 for horse the ratio is 1 to 21. Another comparison of the rate of entry of the species into the two traps is shown by comparing the percentages of the total that were collected in each trap. In the man trap 4.3 per cent of the total were captured, and in the horse trap 95.7. Again this shows a ratio of 1 to 22. A consideration of the percentages of total *albimanus* captured in each trap on each night is indicative of the consistency with which the species entered the horse trap in preference to the man trap. Except for the last few nights of the experiment when the numbers collected were too few to afford reliable comparisons, the highest percentage of the total taken in the man trap was 11, on the morning of March 11. Each night except three the collections in the horse trap were 90 per cent or more of the total collected during the night. Two of the three nights when collections in the horse trap were few were near the end of the experiment when the numbers collected were so low as not to afford reliable expressions.

Considerable variations appear in the numbers of mosquitoes collected during the different nights in both horse trap and in man trap. However, the most pronounced variation is the diminution in numbers collected per night from the beginning to the end of the experiment. This condition is considered to be a result of the cessation of mosquito production in the pond after increasing its salinity in the middle of March by connecting it with the sea. During the experiment notations were made of gross weather conditions and records made of the outstanding features that characterized the weather during the period of observations. The conditions of weather during the period do not seem to influence greatly the entry of the species into the trap nor to influence the ratio of numbers entering the two traps. Certain individual variations however, are apparent. Because of the decreasing numbers encountered per night from the beginning to the end of the observations table 1 is not truly indicative of variations in individual attractiveness, which is shown in table 2.

Table 2 is based on the percentages of the total *Anopheles albimanus* entering the two traps on the nights that each individual served as bait, that were found in the trap occupied by the individual. It is seen that each horse attracted more than 90

TABLE 1  
Comparative attractiveness of humans and horses per *Anopheles albimanus*

DATE, MARCH	BAIT		NUMBER COLLECTED			PER CENT TOTAL		MEAN	WEATHER CONDITIONS
	Man	Horse	Man	Horse	Total	Man	Horse		
2	A. A. W. (W)	A—Red horse	12	364	376	3.2	96.8	188	Normal
3	A. A. W. (W)	A—Red horse	1	285	286	0.4	99.6	143	Normal
4	T. M. H. (W)	A—Red horse	18	346	364	4.9	95.1	182	Normal
5	T. M. H. (W)	B—Dark gray	2	259	261	0.8	98.2	130.5	Cool
8	G. E. B. (W)	C—One eye horse	8	210	218	3.7	96.3	109	Str. br.
9	G. E. B. (W)	B—Dark gray	0	214	214	0.0	100.0	214	Windy, cool
10	P. A. O. (W)	C—One eye horse	10	93	103	9.7	90.3	51.5	Rains, cool
11	P. A. O. (W)	D—Light red horse	16	129	145	11.0	89.0	77.5	Normal
12	A. O. C. (W)	D—Light red horse	19	214	233	8.2	91.8	116.5	Windy
13	A. O. C. (W)	E—Short spotted horse	14	168	182	7.7	92.3	91	Windy, rain
14	H. L. P. (M)	E—Short spotted horse	7	84	91	7.7	92.3	45.5	Wind
16	H. L. P. (M)	F—Old dark red horse	4	106	110	3.6	96.4	55	Str. br.
17	J. E. L. (M)	F—Old dark red horse	1	31	32	3.1	96.9	16	Str. br.
18	J. E. L. (M)	A—Red horse	0	39	39	0.0	100.0	39	Normal
19	E. R. A. (W)	A—Red horse	3	34	37	8.1	91.9	18.5	Wind, rain, cool
20	E. R. A. (W)	B—Dark gray	2	164	166	1.2	98.8	83	Wind, cool
21	19300 (N)	B—Dark gray	2	41	43	4.7	95.3	21.5	Normal
24	19300 (N)	C—One eye horse	1	2	3	33.0	67.0	15	Wind
26	M. C. S. (N)	C—One eye horse	3	6	9	33.0	67	4.5	
27	M. C. S. (N)	C—One eye horse	0	1	1	0.0	100	1	
			Cow						
30	Black cow	D—Light red horse	2	0	2	100.0	0.0	2	
31	Black cow	D—Light red horse	12	1	13	92.3	7.7	6.5	
Totals.....		Man Horse Cow Totals	123 14 137	2791 2791	2928	4.3	95.7	6.1 126.9 7.0	
Mean.....			6.1	126.9	133.0			133.0	

(W)—white, (M)—mulatto, (N)—negro.

per cent of the total for the two traps. The variation in individual attractiveness of different horses based on this analysis is from 91 per cent to 99 per cent of the total. However, there does not appear any definite factor, such as color, that might account for this variation in individual attractiveness. The two horses with which the highest and the lowest percentages were taken were both red in color.

In regard to the variations in the attractiveness of different men it is seen that the variation was

TABLE 2

*Individual attractiveness for Anopheles albimanus\**

HORSE	PER CENT OF TOTAL*
A.....	97
B.....	99
C.....	93
D.....	91
E.....	92
F.....	96
MAN	
A. A. W. (W).....	1.9
T. M. H. (W).....	3.2
G. E. B. (W).....	1.9
P. A. O. (W).....	10.4
A. O. C. (W).....	7.9
E. R. A. (W).....	2.5
H. L. P. (M).....	5.5
J. E. L. (M).....	1.4
19300 (N).....	6.5
M. C. S. (N).....	30.0
Cow.....	93.0

\* Based on the percentages of *Anopheles albimanus* collected in both traps on the nights that the individual served as bait.

(W)—white, (M)—mulatto, (N)—negro.

considerably greater between different men than between different horses. The range in percentages from 1.4 per cent minimum to 30 per cent maximum represents a ratio of 1 to 21. However, the maximum of 30 per cent occurred at the end of the experiment when the numbers caught were too few to offer reliable indications. The next highest percentage was 10.4, which is more than seven times as great as the minimum of 1.4. Again this variation does not seem to be strongly associated with color. The highest percentage, 30 per cent, has already been excluded as probably not being reliable. The next highest, 10.4, and another high

7.9, were with white men serving as bait. One mulatto attracted 5.5 per cent of the total on the nights he served as bait and another mulatto only 1.4 per cent. Excluding the highest percentage of 30, which occurred in collections with a negro, the other negro serving as bait attracted 6.5 per cent of the total. It therefore appears that under the conditions of these observations variations in attractiveness of different people are individual variations, rather than differences in race or color.

#### CONCLUSIONS

Based on the observations described above, *Anopheles albimanus* in Eastern Puerto Rico exhibits a marked preference for horses over man in its selections of a blood meal. The number attracted to horses was more than twenty times as great as the number attracted to men.

The attractiveness offered by different horses for *Anopheles albimanus* appears to be more consistent than that offered by different men, the individual variations between that of different men being comparatively high. For this reason horses are considered to be more satisfactory to use for bait than man in measuring the density of the species within an area. The employment of the standardized human bait catches in this area is therefore of little usefulness for this purpose.

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# IODOCHLORHYDROXYQUINOLINE AND DIODOHYDROXYQUINOLINE: ANIMAL TOXICITY AND ABSORPTION IN MAN.<sup>1</sup>

NORMAN A. DAVID, N. M. PHATAK AND F. B. ZENER

*Portland, Oregon*

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At this time when there is need for a careful appraisal of all available amebacides because of their extensive use by the armed forces, additional experimental and clinical data on such drugs seems appropriate. When iodochlorhydroxyquinoline (Vioform N.N.R.) was introduced as an amebacide it was stated, in summarizing the preliminary experimental work that of some eleven halogenated hydroxyquinolines this drug appeared the most promising for further study, with the possible exception of diodohydroxyquinoline (Diodoquin) (1). Since then, Vioform has been used extensively both as an amebacide and a trichomonacide (2) and, recently, Diodoquin has been recommended for the same purposes (3). However, further experimental chemotherapeutic studies on Diodoquin appear to be lacking except for Tenney's (4) report that no untoward symptoms developed in dogs given oral doses of Diodoquin ranging from 4.0 to 6.0 grams per day for a period of six days. We wish, therefore, to present the results of two comparative studies made recently on Vioform and Diodoquin. The first investigation offers data comparing their toxicity in guinea pigs and kittens. The second study was carried out to provide information relative to the absorption and excretion of these compounds in the human and comprises a determination of the blood iodine values before and after administration of these two amebacides.

## ANIMAL TOXICITY

**Methods.** Weighed amounts of the powdered drug enclosed in capsules were administered orally to the animals. The animal was held and observed until all of the several capsules containing the dose were swallowed. The guinea pigs weighed from 300 to 600 grams and were about two months old and the kittens weighed from 400 to 900 grams and were from six to eight weeks old. Control animals

kept in adjoining cages remained healthy and alive during the observation period. Animals dying from the effects of the drugs were autopsied and specimens taken for histological examination.

**Results.** 1. Guinea pigs. The results of the toxicity studies on both guinea pigs and kittens are shown in table 1. In a previous investigation (5) as well as in the present study Vioform was found to kill 7 of 10 guinea pigs when given in single oral doses of 200 mgm. per kilogram. In doses above 300 mgm. per kilogram Vioform consistently killed a majority of the animals. From these results, the LD<sub>50</sub> for Vioform in the guinea pig is estimated to be about 175 mgm. per kilogram. With Diodoquin, however, no definite toxic effect at any certain dosage was observed and the lethal dose (LD<sub>50</sub>) could not be established. Small doses (50 and 100 mgm. per kilogram) of Diodoquin given to two separate groups of five animals killed 4 of the 10 treated pigs while larger amounts (up to 2.0 grams per kilogram) failed to show an expected increase in toxicity. Although various doses of Diodoquin were given to one hundred and forty animals divided into sixteen separate groups, it was found that as many animals died from the small doses as from the larger amounts. The highest toxicity observed for Diodoquin was with a dose of 300 mgm. per kilogram which killed 4 of 10 guinea pigs.

2. Kittens. Vioform killed 2 of 7 kittens given a single oral dose of 300 mgm. per kilogram. The LD<sub>50</sub> of Vioform in kittens is probably larger than the dose we used but further studies were not done because of lack of animals. Although a greater number of kittens were given Diodoquin, the results were again inconsistent. Doses of 300 and 500 mgm. per kilogram of Diodoquin killed 2 of 14 and 2 of 16 animals, respectively, but a dose of 750 mgm. per kilogram was withstood by seven other kittens.

Histologic postmortem examination of animals dying after single oral doses of Vioform and Diodoquin showed some liver damage. This injury was similar to that previously reported for rabbits

<sup>1</sup> From the Department of Pharmacology, University of Oregon Medical School, Portland, Oregon. Supported, in part, by a grant from Ciba Pharmaceutical Company, Ind., Summit, New Jersey.

dying from Vioform (1) and is of the same type as seen with chloroform poisoning.

IODINE ABSORPTION

Absorption and excretion of the iodine molecule of Vioform occurs in man since iodine can usually be found in the urine after the drug has been taken

assumed that absorption is possible after oral administration under certain conditions. If absorption of Diodoquin occurs, there should be an increase in the blood iodine. We undertook to investigate this possibility by studying the blood iodine changes in healthy human subjects given therapeutic doses of either Vioform or Diodoquin.

TABLE 1  
*Oral toxicity of single doses of vioform and diodoquin in guinea pigs and kittens*

DRUG	ANIMAL	PLACE STUDIED	DOSE  mg./kg.	MORTALITY		TIME OF DEATH  days
				Number died	Number used	
Vioform	Guinea pigs	California	200	7	10	4, 4, 4, 5, 5, 6, 7
			250	9	10	2, 3, 4, 4, 4, 5, 5, 5, 6
		Oregon	300	10	10	all in 5
			150	2	10	1, 3
			175	5	8	6, 6, 6, 9, 17
		Kittens	200	7	10	3, 3, 4, 4, 4, 4, 4
			250	1	8	20
			300	0	7	
			350	2	7	22, 24
Diodoquin	Guinea pigs	California	50	2	5	13, 16
			100	2	5	5, 5
			200	1	10	11
			300	4	10	2, 5, 10, 11
			400	2	10	2, 14
			500	0	5	
			600	1	10	11
			800	0	10	
			1,000	0	10	
		Oregon	600	1	10	15
			750	0	9	
			1,000	0	10	
			1,200	1	10	10
	Kittens	Oregon	1,500	2	7	4, 7
			1,500	2	9	19, 21
			2,000	2	10	10, 11
			300	2	14	10, 12
			500	2	16	17, 19
			750	0	7	

for several days. For Diodoquin, however, the statement has been made that "the drug is recovered quantitatively in the stools and even with enormous dosage, no absorption takes place" (6). Since we found Diodoquin to be occasionally lethal in animals and capable of causing liver damage, we

*Methods.* Blood iodine was determined by Stevens' (7) method which we had found satisfactory in other studies (8). A blood sample for analysis was taken before breakfast the day treatment was started and again the day after concluding therapy. Diodoquin was given in a dosage of 0.21 grams (1

tablet) three times a day for ten days to 8 medical students and 2 laboratory workers. Vioform capsules of 0.25 gram were given three times a day for the same period to 9 other male students. Both drugs were taken after meals. The total dosage of Diodoquin (6.3 grams) for the ten day course was less than that of Vioform (7.5 grams). All sub-

jects kept a daily record of any untoward symptoms noted.

Most of the subjects noted some gastrointestinal discomfort during the time they were taking these drugs. None of the complaints, however, were severe enough to warrant discontinuing medication for more than one or two doses. A number of pa-

TABLE 2  
*Blood iodine changes and symptomatology following treatment with vioform and diodoquin*

DRUG AND DOSAGE	PATIENT	BLOOD IODINE (MICROGRAMS PER 100 CC.)			SYMPTOMS NOTED
		Normal	End of treatment	Increase	
Vioform, 0.25 g. T.I.D. for 10 days	J. E. S.	7.42	152.6	145.18	Mild pruritis ani last 3 days
	B.-H.	13.78	161.1	147.32	Anal pruritis from 7th to 30th day
	R.-R.	40.28	228.9	188.62	Occasional "heartburn," nausea
	H. L. F.	9.54	230.2	220.66	None
	R. L. R.	6.99	243.8	236.81	Sl. diarrhea on 5th and 6th day; anal irritation
	J. M. P.	13.78	251.2	237.42	Some "gas" and abdominal distension
	J. R. H.	8.48	260.7	252.22	Pruritis ani from 2nd day to 14th day
	E. G. C.	8.48	262.88	254.40	Pruritis ani last 7 days
	C. W. D.	11.66	339.28	327.62	Sl. gastric irritation first 3 days
	Average .....			223.36	
Diodoquin, 0.21 g. T.I.D. for 10 days	D.-McK.	11.6	57.2	45.6	Sl. "gastric" distension and irritation
	R. E. S. (Mrs.)	18.02	87.98	69.96	Sense of increased warmth
	J. H. G.	7.42	77.38	70.96	Lethargy and constipation
	F. L. C.	10.6	85.86	75.26	Several attacks mild diarrhea and anal irritation
	F. B. Z. (Dr.)	11.66	111.30	99.64	Lethargy; Headache; Sense of warmth
	R. L. O.	18.02	112.63	104.61	Anal pruritis, mild, from 4th to 10th day
	G. D. S.	5.3	181.72	176.42	None
	F. B. M. (Mrs.)	5.3	284.37	279.07	Mild pruritis ani
	J. W. R.	10.38	374.18	363.80	Rhinitis last 3 days; Sl. anal irritation 10th day
	S. B. McK.	9.01	446.26	437.25	Sl. gastric "heaviness" first 3 days
	Average .....			172.25	

jects kept a daily record of any untoward symptoms noted.

Table 2 shows that a greater average rise in blood iodine (223.36 micrograms) occurred after treatment with Vioform than after Diodoquin (172.25 micrograms). Individual variations in the increase in the blood iodine level were greater with Diodoquin with rises from 45.6 to 437.25 micrograms per 100 cc. of blood being observed. More consistent absorption of Vioform was noted where

tients experienced a moderately severe pruritus ani which had developed on about the fourth or fifth day of treatment and persisted for several days after the drug was stopped. No symptoms suggestive of iodism were observed.

#### COMMENT

The previous study (5) on Diodoquin was dropped and the results not published mainly because a definite LD<sub>50</sub> could not be determined in



spite of the use of a large number of animals. It was assumed that a compound of this nature, which exhibited such irregularities in toxicity, would also prove uncertain in protozoacidal action.

Diodoquin is extremely insoluble in water, dilute acids and alkalis. Consequently, it might be expected to remain stable and unabsorbed in the gastrointestinal tract and thus show little or no toxicity after oral administration. We found, however, that single oral doses ranging from 50 to 2000 mgm. per kilogram did kill some animals. While we could not determine the  $LD_{50}$  for Diodoquin in the guinea pig, we do not believe that our results would be appreciably altered by further studies on a larger number of animals. Too few kittens were given Diodoquin to permit any conclusions to be made.

The toxicity of an insoluble type of compound such as Diodoquin may depend more on such factors as hyperacidity or intestinal stasis than on the actual amount of drug administered. For example, Anderson and Reed (9) believe that hyperacidity can cause an increase in the toxicity of Vioform in man. They state that they had observed a patient who could not tolerate this drug because a soluble hydrochloride of Vioform had apparently formed "in the excess acid present in the patient's stomach, which resulted in greater absorption of a more toxic compound." When such conditions are encountered, the potential danger of toxicity should be greater when Diodoquin (M.W. 396.9) is employed therapeutically since it contains 63.9 per cent iodine compared with the 41.5 per cent of iodine present in Vioform (M.W. 305.4). It has been shown also that toxicity increases with halogenation of oxyquinoline and in proportion to the atomic weight of the halogen present (10). Apparently, the relative insolubility of Diodoquin is a confusing factor in toxicity studies so that it can not be predicted whether or not a certain dose will kill.

In our blood iodine studies the total amount of Diodoquin given was considerably less than that recommended for the treatment of amebiasis. The average adult dose of Diodoquin is 7 to 10 tablets daily, taken between meals for fifteen to twenty days. This increased dosage compared with the amount we administered, and the prolongation of treatment would undoubtedly increase the danger of adsorption and toxicity from Diodoquin. If a drug is an effective protozoacide, we believe that nothing is gained by continuing treatment for

periods longer than ten days at a time. The unreliability and irregularity of the absorption of Diodoquin after oral administration is in our opinion a major handicap for its uncontrolled therapeutic use. Our results indicate that iodine absorption does occur after Diodoquin, as well as Vioform, when it is given orally and that both drugs are capable of causing toxicity in animals. Craig (11) has recently suggested that Diodoquin could be used in full therapeutic dosage for prolonged periods in the prophylaxis of amebiasis. On the basis of our results we believe that Vioform would prove a more reliable protozoacide and less likely, due to less variable absorption, to cause toxic symptoms. The use of either of these compounds in the prophylaxis of amebiasis must be rigidly controlled and should not be carried out as extensively or as freely as is done in the prophylaxis of malaria with quinine. However, we feel that any procedure, such as Diodoquin or Vioform prophylaxis, intended to safeguard the health of troops and workers in ameba infested areas is worth the risk of the occasional case of toxicity to iodine which may appear.

#### CONCLUSIONS

1. The oral  $LD_{50}$  for Vioform is about 175 mgm. per kilogram in the guinea pig and approximately 400 mgm. per kilogram in kittens. No definite  $LD_{50}$  was established for Diodoquin but it proved lethal to both guinea pigs and kittens in occasional instances when doses from 50 to 2000 mgm. per kilogram were used.
2. Liver damage was observed in animals dying from Diodoquin or Vioform.
3. Iodine absorption occurs in normal human subjects when either Vioform or Diodoquin are administered orally in therapeutic amounts for a period of ten days. This is demonstrated by a rise in blood iodine which shows greater variation with Diodoquin than with Vioform.
4. Diodoquin, because of its higher iodine content, possesses a greater potential danger of toxicity than Vioform.
5. The occasional toxicity which may be seen in the human following oral administration of these drugs should not preclude their use in the prophylaxis of amebiasis if such therapy is rigidly controlled.

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# THE ADAPTATION OF A CANE RAT (*ZYGODONTOMYS*) TO THE LABORATORY AND ITS SUSCEPTIBILITY TO THE VIRUS OF YELLOW FEVER<sup>1</sup>

MARSTON BATES AND JOHN M. WEIR

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A special effort has been made in the past few years to domesticate various rodent species found in the vicinity of the Yellow Fever Laboratory at Villavicencio, Colombia, with the object of testing their value in laboratory studies. This is a continuation of work started by Dr. John C. Bugher and his colleagues, who have published a general description of the region and of the methods of work (Bugher *et al.*, 1941). The local cane rat has proven especially well adapted to the laboratory, and the present paper includes general observations on its laboratory behavior and an account of experiments on its susceptibility to the virus of yellow fever.

We are indebted to Dr. G. H. H. Tate of the American Museum of Natural History for the identification of the animal, and to Miss Barbara Lawrence of the Museum of Comparative Zoology for searching the literature for notes on the behavior of this and other mammals; study specimens of our experimental material have been deposited in both museums.

## BIOLOGICAL NOTES

The Villavicencio cane rat seems to be very similar to the animal described from Maipures, Venezuela, by Thomas, as *Zygodontomys stellae*, now generally considered to be a subspecies of *Z. microtinus*. In view of our limited knowledge of the range and geographical variation of the rodents of northern South America, the Villavicencio population of this rat is perhaps best referred to as *Zygodontomys microtinus* near *stellae* Thomas (for taxonomy and bibliography cf. Tate, 1939). The cane rat here, like its relatives in Panama (for life history notes on the similar Panama cane rat cf. Enders, 1935; Bole, 1937) seems to be especially common in semicultivated land, and it is abundant in the abandoned pastures around the laboratory.

<sup>1</sup> The studies and observations on which this paper is based were conducted with the support and under the auspices of the Section of Special Studies maintained by the Ministry of Labor, Hygiene, and Social Welfare of the Republic of Colombia and the International Health Division of The Rockefeller Foundation.

We found that it would breed readily in captivity, and when preliminary tests showed it that might possibly be of use in influenza studies, a laboratory colony was established. All experimental work was done with material from this colony, which was descended from six wild males and twelve wild females.

We have maintained the colony at a level of fifty breeding females and thirty-five males; from these we get from fifty to eighty young a month, which is adequate for our needs. The gestation period seems to be about twenty-eight days, rats reach sexual maturity in three to four months, and the average litter contains 4.0 young (means of 100 litters). The young can be weaned at fifteen days, but we generally leave them with the mother for somewhat longer—seventeen to twenty days. The females may be impregnated again as soon as the young are born; but such frequent parturition results in progressively weaker litters, so our standard practice is to remove females as soon as they are obviously pregnant, and not mate them again until after the young are weaned.

The cane rat colony is maintained on the following diet:

	Part
Crushed carrots.....	1
Crushed cabbage.....	$\frac{1}{2}$
Maize flour.....	1
Rice polishings.....	$\frac{1}{2}$
Rolled oats.....	1
Water.....	2
Boiled milk.....	1
Calcium carbonate.....	0.05
Sodium chloride.....	0.05
Vegetable lard.....	0.01

The mixture has the consistency of paste, which makes it easy to handle. The rats are given all they will eat, about 20 grams per adult rat per day, and on this they seem to thrive with no supplementary food and no water. This diet has proven to be quite unsatisfactory for our white mice, however, and is inadequate for some other species of wild rats.

The cane rat is of medium size (mean weight of

ten adult males, 82 grams). It is a very active species, given to making long and sudden leaps, causing difficulty in handling in the laboratory. We make a practice of anesthetizing the rats one at a time when they are being used for experimental work, as they are easily killed by an overdose of ether. With practice and good luck, it is possible to get 2.0 cc. of blood from the heart of adult rats, but we have never succeeded in bleeding an animal without killing it. In susceptibility to ether and to heart puncture, this species is much more delicate than some of the local rodents. No spontaneous diseases have appeared in the colony, and mortality from accident is low.

#### EXPERIMENTS WITH YELLOW FEVER VIRUS

*Recovery of virus.* We have not been able to recover virus from these animals after either subcutaneous or intraperitoneal inoculation with virus of the neurotropic strain, or with either of two strains of local virus. Attempts have been made with fourteen animals, inoculating blood, liver, and brain material taken at intervals between four and seven days after infection intracerebrally in susceptible white mice. There has been no mortality that could be attributed to yellow fever after either subcutaneous or intraperitoneal inoculation.

The virus seems invariably to grow in brain tissue after intracerebral inoculation, but as in the case of some strains of white mice (Sawyer and Lloyd, 1931) the animals do not invariably die. We have always found virus in the brains of animals infected with the neurotropic strain and killed between the fourth and seventh days after inoculation; three of four killed on the eighth day showed virus; one killed on the tenth day showed no virus.

*Serial passage of virus.* The "French neurotropic" strain of virus was carried through twelve intracerebral passes in these animals, as summarized in table 1. These passes were carried out by the same method as used routinely for white mice: 0.03 cc. of brain suspension was inoculated in the right cerebral hemisphere of each animal. The brains used for inoculum were normally ground in 3 cc. physiological saline each (giving a suspension of approximately 20 per cent by weight), pooled, and centrifuged for fifteen minutes at 1,500 r.p.m. It will be seen that the third, seventh, and eighth passes were carried out by the inoculation of brain material from apparently

healthy animals. From these and other experiments, it appears that the virus invariably grows to some extent in the brain tissue, though the growth may be slight, as shown by the titrations

TABLE 1

*Summary of intracerebral passes of neurotropic virus in cane rats*

PASS NO.	INOCULUM		WHITE MOUSE CONTROLS A.S.T.	NO. OF RATS INOCULATED	AGE OF RATS	DAY OF FIRST PARALYSIS	NUMBER DYING
	Animal source	Days after inoculation					
1	"Neurotropic" Mouse Pass 629		4.6	5	70	7	3
2	2 rats S†	7	5.3	4	25	x	0
3	2 rats N‡	8	6.6§	5	32	6	3
4	2 rats S	6	4.6	9	48-54	5	6
5	1 rat S	5	5.5	10	46-78	5	2
6	2 rats S	5	5.5	5	70	7	3
7	4 rats N	5	x¶	2	40	x	x
8	2 rats N	6	6.4	7	43	7	5
9	2 rats S	6	4.7	9	42-49	6	4
10	2 rats S	6	5.3**	6	48	5	6
11	2 rats S	5	4.7	8	38-43	5	5
12	2 rats S	5	4.8	10	36-38	5	4

\* A.S.T. = average survival time of white mouse controls on each inoculum.

† S = paralyzed animals.

‡ N = apparently healthy animals.

§ Two normal rats killed on eighth day; brain of one gave 2/12 mortality in white mice, of the other 12/12 mortality with A.S.T. of 6.6 days. Pool of the two brains used as inoculum for third pass.

|| A.S.T. for a 20 per cent suspension by weight in saline, undiluted; this inoculum showed a titer of 1:50,000 in white mice.

¶ Four apparently healthy rats killed on fifth day; the brains ground separately (10 per cent suspension by weight in serum-saline) showed the following titers: 1:5, 1:315, 1:1.6, 1:2.2. Pool of supernatants used as inoculum for seventh pass.

\*\* Ten per cent suspension in serum-saline showed a titer of 1:23,000 for one animal, 1:31,000 for the other; pool used as inoculum.

of the four rats used as the inoculum for the seventh pass.

There is some evidence in this series of adaptation of the virus to this species of rat. The number of rats used for each pass is too small for the computation of mortality rates, and since the

animals used in a particular pass were frequently siblings, the genetic composition of the particular family used for a pass undoubtedly influenced the result. These difficulties can be avoided to some extent by grouping the passes as follows:

PASSES	TOTAL ANIMALS	NUMBER DYING	MORTALITY
			<i>per cent</i>
1 to 3	14	6	43
4 to 6	24	11	46
7 to 9	16	9	56
10 to 12	24	15	62

In this summary, as in the table, animals killed when paralyzed are considered as "dying" and animals killed when normal, as "living." We

TABLE 2

*Protection test results with sera taken 30-60 days after inoculation*

VIRUS STRAIN	INOCULATION ROUTE	PROTECTION TEST RESULTS		
		Negative	Doubtful	Positive
"Neurotropic"	s.c.	6	1	4
	i.p.	0	0	1
	i.c.	1	1	4
"Novoa" 228 m.l.d.	i.p.	0	0	2
	i.c.	0	0	2
"Novoa," trace ( $\pm$ 1 m.l.d.)	i.c.	3	0	0
"17D" (vaccine)	i.p.	2	0	2

have not observed any case in which an animal has recovered after showing frank paralysis.

*Antibody production.* Sera were tested for yellow fever antibodies using the intracerebral protection test as described by Bugher (1940). Sera of some fifteen uninfected animals were tested for antibodies, and all gave clear negative results. It thus seems unlikely that there is a nonspecific reaction in this species. Protection tests on infected animals are summarized in table 2. From

this, it appears that the animal generally, though not always, develops antibodies after intracerebral or intraperitoneal inoculation with appreciable doses of virus.

## SUMMARY

The Villavicencio cane rat has been found to be well adapted to laboratory culture and manipulation.

The virus of yellow fever seems to grow readily in the brain tissue of this animal, but only about half of the animals are killed. Virus was successfully maintained for twelve serial passes and showed some signs of adaptation in that the rat mortality became successively higher and the incubation period shorter.

Circulating virus was not recovered after subcutaneous or intraperitoneal inoculation, but many animals showed circulating antibodies after such inoculation as well as after intracerebral inoculation as shown by the intracerebral protection test in white mice.

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# COMPARATIVE MORPHOLOGY OF THE TRICHOMONAD FLAGELLATES OF MAN

D. H. WENRICH

Department of Zoology, University of Pennsylvania, Philadelphia

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## INTRODUCTION

Recent papers (28, 26, 27) indicating that trichomonad flagellates from the human intestine and mouth are unable to establish themselves when implanted into the human vagina, along with much additional evidence, has served to emphasize the separateness of the species of *Trichomonas* located in the vagina, the mouth, and the intestine, respectively.

In spite of many excellent morphological studies on these species, especially on *T. vaginalis*, many textbooks on parasitology continue to state that morphologically the three species are identical. Other authors declare that the intestinal and vaginal forms are identical or that the intestinal and oral forms are the same.

For more than 20 years I have been interested in the morphology of trichomonad flagellates and have recorded my observations on those found in man in a number of publications either with (3, 4, 6) or without (31, 32, 33) collaborators. A paper on comparative morphology (31) was published in South America and one on the morphology of *T. vaginalis* (33) in Japan. Since these papers are not as generally available as could be desired and since my studies have recently brought to light some previously unrecorded details of organization, it seems worthwhile to present an account of the morphological characteristics of these species. In the interest of greater completeness a review of recent literature showing physiological distinctions among these species is included. A more extended description of *T. hominis* is being published elsewhere (35), and an abstract on comparative morphology has been published (34). Since the literature dealing with the trichomonad flagellates of man is so voluminous, only a selected list of references will be cited.

**Materials and methods.** Living flagellates as well as those on fixed and stained slides have been studied. Living animals were examined in freshly obtained body discharges diluted with physiological salt solution, or in the fluid media in which they

were cultured. Prepared slides from diluted feces or vaginal secretions were made by fixing in Schaudinn's, Bouin's, Hollande's or Flemming's fluids, Chrom-acetic, etc., with various modifications, and stained for the most part with Heidenhain's iron haematoxylin. Smears of culture flagellates or of vaginal discharges were treated with Osmic fumes, dried, fixed with methyl alcohol and stained by the Giemsa method.

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## COMPARATIVE MORPHOLOGY AND BEHAVIOR

In the presentation immediately following, *Trichomonas vaginalis*, *T. tenax* (*T. elongata*, *T. buccalis*) and *T. hominis* (*T. intestinalis*, *Pentatrichomonas arindellei*) alone will be considered, since these are the species commonly observed. *Tritrichomonas fecalis* Cleveland has been reported only once in the history of human parasitology and will be dealt with separately. Reasons for employing the names here used are given in the discussion.

**Size and shape.** Unless otherwise stated, the measurements given below are based upon fixed and stained specimens. Living animals are regularly larger than those on prepared slides. The recorded lengths do not include the flagella nor the protruding portion of the axostyle.

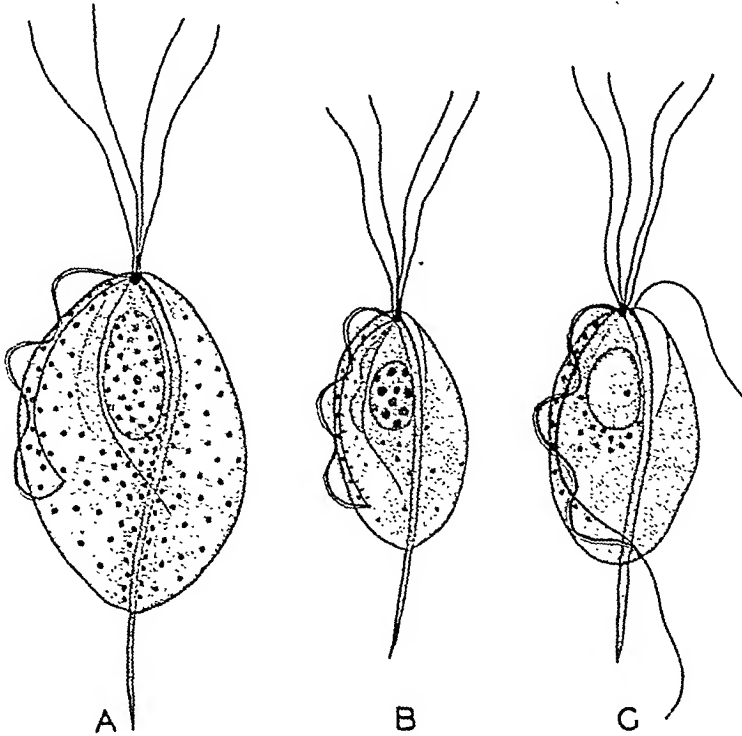
In the present studies, lengths of *T. vaginalis* ranged from 7 to 23  $\mu$ , with an average of about 13  $\mu$ . Most specimens fell within the range from 12 to 20  $\mu$ . Living individuals up to 30  $\mu$  long have been noted. Lengths of *T. hominis* ranged from 5



to 14  $\mu$  with averages in different groups from 7 to 8  $\mu$ . Measurements of *T. tenax* gave a range from 5 to 12  $\mu$  with averages for different groups from 6.5 to 7.5  $\mu$ . Thus, *T. hominis* and *T. tenax* are about the same length, the former being slightly longer.

All three species show, in general, the fusiform or piriform shape common for species of *Trichomonas* (textfigs. A, B, C). On stained slides *T. hominis* is generally more strongly arched on the dorsal profile (fig. 11). All three are characterized by great protoplasmic plasticity so that various shapes may be assumed. *T. vaginalis* is usually

uncertain. In *T. vaginalis* and *T. tenax* the blepharoplast is commonly a single body or a cluster of closely adherent granules, but sometimes it appears to be composed of two approximately equal parts. In *T. hominis*, in the more common five-flagellated condition, there are characteristically two blepharoplasts of unequal size at the head of the axostyle. The smaller, ventral one gives rise to the single ventral flagellum, while the more dorsal, larger one gives origin to the other flagella and the costa (figs. C, 13). It will, of course, be appreciated that only favorably oriented individuals,



TEXTFIGURES A, B AND C. DIAGRAMMATIC REPRESENTATIONS OF *TRICHOMONAS VAGINALIS* (A), *T. TENAX* (B), AND *T. HOMINIS* (C)

more robust in appearance (figs. 3, 4) and *T. tenax* is commonly more slender (figs. 7, 9). In the rounded-up, inactive, condition, *T. vaginalis* tends to assume a spherical form while *T. hominis* is more often oval. These inactive stages are considered to be degenerate, or possibly abortive attempts at encystment. True resistant cysts have not been satisfactorily demonstrated.

**The blepharoplasts.** The fibrillar apparatus of trichomonads centers in the blepharoplastic complex made up of a group of basal granules more or less fused together at the anterior pole of the cell. The exact number of component basal granules is

especially those presenting a side view, could be expected to show the two elements distinctly.

**The anterior flagella.** Four anterior flagella, of approximately equal length, are characteristic for *T. vaginalis* and *T. tenax*. They are sometimes grouped into two groups of two each, one pair which may be a little longer than the other. On individuals of *T. vaginalis* of medium size the flagella are about as long as the body. On larger animals they may be shorter, and on shorter animals they may be longer, than the body. On *T. tenax* the latter tendency is more pronounced (Figs. 9, 10). Occasionally on stained slides one sees a

specimen of *T. vaginalis* or *T. tenax* with three or five flagella, but these are rare. On *T. hominis* the number of anterior flagella is notably variable, three, four or five being commonly reported. In all the 20 cases which I have studied (four other cases had too few flagellates to study), some, or most of the individuals showed five anterior flagella; and also the two unequal blepharoplasts. Counts from Giemsa-stained clone-culture smears showed a majority of animals with five flagella. However, some other populations seemed to have a majority with four. In my experience, therefore, the number of anterior flagella on *T. hominis* is variable, but more characteristically, five. The anterior flagella of this species show a greater tendency to inequality in length among themselves than is true of the other two species. The single ventral flagellum may be as long as the other four (fig. 12) but is more commonly shorter (fig. 13).

*The posterior flagellum and the undulating membrane.* On *T. vaginalis* the undulating membrane is usually between one-third and two-thirds the length of the body, ending where the posterior flagellum becomes attached. This flagellum has no posterior free extension. In very large specimens the membrane is relatively short (fig. 4), while in very short individuals, especially those reduced in size by autotomy, the membrane may be relatively much longer and even full-length.

As pointed out elsewhere (33), the margin of the membrane has two filaments, the posterior flagellum, and an accessory filament of finer caliber (fig. 4). This accessory filament is not commonly visible on the customary wet-fixed and stained preparations (figs. 1, 2, 3, 5), but on dried films, dyed with Giemsa's stain, it is often separated from the posterior flagellum (fig. 4). In such preparations it is somewhat longer than the flagellum, possibly due to a capacity to swell or to more shrinkage on the part of the flagellum.

The undulating membrane of *T. tenax*, like that of *T. vaginalis* is characteristically less than body-length and the posterior flagellum has no free extension. There is an accessory marginal filament (fig. 10). Possibly associated with the smaller size of body, the undulating membrane of *T. tenax* is relatively somewhat longer than on *T. vaginalis* (figs. B, 7-10). In some cases, perhaps associated with autotomy, the membrane may reach to the posterior end (fig. 9).

The posterior flagellum of *T. hominis* passes along the margin of the undulating membrane and

then projects beyond the body as a trailing flagellum almost as long as those at the anterior end (figs. C, 11, 12, 14). There is an accessory filament of finer caliber which normally ends at the posterior end of the membrane, and on Giemsa-stained dried smears it is peripheral to the posterior flagellum (fig. 12). For some animals on these dried smears the cytoplasm was destroyed leaving only the blepharoplast with the structures which are attached to it, and sometimes the nucleus also. Such specimens occasionally showed the accessory filament flattened out into a "fin" attached to the posterior flagellum (fig. 13). In a few cases this "fin" became greatly expanded to become longer than the posterior flagellum itself (35).

*The costa.* The chromatic basal rod, or costa, is located beneath the undulating membrane. In *T. vaginalis* (figs. A, 1-5) and *T. tenax* (figs. B, 7, 8, 10) this rod is very slender and is uniform in diameter throughout its length. It is only as long as the undulating membrane and therefore is characteristically less than body-length in these two species. In *T. hominis* the full-length costa is much coarser and is thicker through its midregion (figs. C, 14).

*The parabasal apparatus.* Both *T. vaginalis* and *T. tenax* have a well-developed parabasal apparatus consisting of a slender parabasal fibril and a much thicker and shorter parabasal body (figs. A, B, 2, 3, 4, 8). The fibril may be observable after the more common fixing and staining methods but the parabasal body is rendered more stainable by fixing agents containing Osmic or Chromic acid, such as Flemming's fluid or Chrom-acetic. Occasionally it is visible on dried smears treated with Osmic fumes and stained by the Giemsa method (fig. 4). The appearance of the parabasal body may vary in different individuals. In *T. vaginalis* it usually presents a uniform diameter except for the narrower attachment to the blepharoplast, and it curves gently over the nucleus like a link of sausage, lying between the nucleus and the costa (fig. A). Occasionally it may show a constriction near its own mid region where it may also be rather sharply bent (fig. 2). Sometimes the dorsal margin shows several irregularities. In some populations a number of individuals possessed two parabasal bodies, one shorter than the other (fig. 3). The smaller one may be growing out in anticipation of cell division. In very large individuals the fibril may be quite long, reaching to the posterior end of the body and occasionally it may be split (33).

In *T. tenax* the parabasal body is variable in length and form. Sometimes it is short and biscuit-shaped (31) but is often longer. It may show irregularities in its dorsal contour (fig. B) and inequalities in stainability in different regions (fig. 8).

*T. hominis* differs radically from the other two species in the apparent absence of a parabasal body. Repeated attempts to demonstrate it have been made, using the same techniques employed for the other species, but without success. However, as shown in figures 11, 13 and 14, one may often see a slender fibril or a row of fine granules between the nucleus and the costa where the parabasal apparatus should be, if present; but this structure could be a new costa growing out in anticipation of cell division. The absence of a parabasal body is a striking distinction between *T. hominis* and the other two species.

*The axostyle.* The axostyles of all three species are relatively solid, tapering gradually from within the body to the posterior tip and lacking endoaxostylar granules (figs. A, B, C). That of *T. hominis* is relatively coarser (fig. C). In *T. vaginalis*, especially in large individuals, the axostyle may be split up into a number of threads in the region posterior to the nucleus (fig. 4). On the slide from which figure 4 was drawn a great majority of the individuals had split axostyles. Many other observers have noted this phenomenon. In all three species the ventral margin of the anterior region of the axostyle, which serves as the "roof of the mouth" is thicker and stains more intensely than elsewhere (figs. A, B, C, 1, 7, 11, 13).

*The nucleus.* In *T. vaginalis* the nucleus is commonly elongate-oval (figs. A, 5), or even quite long and narrow, but sometimes is more rounded (fig. 1). In the other two species it is more commonly rounded or oval (figs. B, C). In *T. vaginalis* the chromatin, aggregated into small granules, is usually rather uniformly distributed throughout the nuclear space (fig. A). In *T. tenax* the granules tend to be fewer and larger (figs. B, 7). In *T. hominis* the chromatin is less often aggregated into granules and tends to form a layer against the nuclear membrane (fig. C). In all three species the endosome is relatively small and often difficult to see, especially in *T. vaginalis*. It is usually more conspicuous in *T. hominis*. In the latter species the nucleus may be parasitized by *Nucleophaga* (fig. 14), but I have never seen this parasite in the other two species.

*The cytoplasmic granules.* Siderophilic cyto-

plasmic granules are found in many species of *Trichomonas* with arrangements which are specifically characteristic. They do not usually appear in the Giemsa-stained smears. *T. vaginalis* differs from the other two species because of the large number of such granules. They are more numerous along the axostyle and costa (figs. A, 1-3, 5). In *T. tenax* they are much less conspicuous and appear along the costa but apparently are not associated with the axostyle (figs. B, 7, 8). In *T. hominis* there is a row close to the costa (figs. C, 11, 14). It is probable that some, at least, of the paracostal granules in *T. vaginalis* and *T. tenax* are homologous with the paracostal row in *T. hominis*. In the latter species there are also perinuclear granules, which are difficult to see unless one looks for them (figs. C, 14). They are irregular in distribution and are sometimes replaced by a chromatic cloud (35).

*The cytostome.* In *T. vaginalis* the cytostome is inconspicuous although readily apparent in some specimens (figs. A, 1, 2). In *T. tenax* it is more commonly visible (figs. B, 7, 8) and in *T. hominis* may be quite conspicuous (figs. C, 11). It is always at the anterior end on the side of the axostyle away from the undulating membrane. The presence of a mouth would presuppose that ingestion is accomplished through its use, although the suggested possibility of ingestion by pseudopodia (13, 25) cannot be excluded.

*Behavior.* In behavior the three species are somewhat similar as seen at room temperature in physiological salt solution or in liquid culture medium. Under these conditions, *T. hominis* and *T. tenax* are usually more active. In each species the body shows great plasticity enabling the flagellates to burrow through the loose debris in their environment. *T. vaginalis* has a more marked tendency to adhere to solid objects by the sticky posterior cytoplasm and one often sees clusters of individuals adhering together, or to leukocytes or other objects. The anterior flagella and undulating membrane of attached animals beat vigorously causing them to rotate on their long axes. Individuals progressing across the field of the microscope often become adherent to the substrate by the posterior cytoplasm. This may become pulled out into long strands which then break and permit continued forward progression. *T. tenax* resembles *T. vaginalis* in these characteristics more than does *T. hominis* which I have seldom seen in clusters or adhering to solid objects.

*T. vaginalis* shows pseudopodial activity more frequently than do the other two species and this behavior has been observed by various authors. Under certain conditions, clear pseudopodia, comparable to those of amoebae, are slowly protruded from different parts of the body, but especially from the posterior region (fig. 5). Such pseudopodia are rare in *T. hominis* but have been reported for *T. tenax*. (13).

Under conditions considered degenerate or preliminary to rounding up, the flagella of trichomonads may become adherent to the cell body. These imbedded flagella continue activity for some time and this may result in the rapid extrusion from the anterior region of long finger-like processes which may then progress, with diminishing amplitude, to the posterior end. Such protrusions have been mistaken for pseudopodia, but are thrust out much more rapidly and vigorously than true pseudopodia.

#### DISCUSSION

*Comments on certain morphological features.* Some features of morphology may profitably be discussed with reference to reports in recent literature.

The great range in size sometimes seen, especially for *T. vaginalis*, is a matter of considerable interest. The lower size ranges of this species overlap the sizes of the others. Larger dimensions have been reported for *T. tenax* in cultures, with maximum lengths of 21  $\mu$  (20), and 18  $\mu$  (23). Various suggestions have been made as to the cause of these variations. Size races could be represented. Rapid multiplication with little growth between divisions would result in smaller animals. Greater growth and fewer divisions would produce larger animals. Larger sizes of *T. vaginalis* have been thought to be due to "overgrowth" (37). *T. tenax* was found to increase greatly in size when rice starch was added to the culture medium (23, 2), as was also noted for *T. hominis* (11). In bacteria-free cultures *T. vaginalis* became somewhat larger when the pH varied above or below the optimal range from 5.15 to 5.80 (24). Autotomy may account for reduction in size. In this process, which has been described for *T. tenax* (19) and for *T. vaginalis* (25), a blob of cytoplasm becomes constricted off and detached from the posterior end (fig. 6). With so many factors influencing size it is difficult to evaluate the ranges that have

been observed. However, *T. vaginalis* is characteristically larger than the other two species.

Cytoplasmic inclusions are of some interest. In fresh material *T. vaginalis* rarely shows inclusions except a leukocyte in an occasional individual (31, 3, 4). On the other hand, this species may be phagocytized by leukocytes (8, 31, 4) as is also true of *T. hominis* (10, 31, and fig. 17). There are many reports of the ingestion of erythrocytes by *T. hominis*; and also by *T. vaginalis* in culture (1, 25). In cultures, all three species ingest bacteria; *T. tenax* readily ingests starch (23, 2) as does also *T. hominis* (11) and *T. vaginalis* (25). Intestinal trichomonads are sometimes parasitized by *Sphaerita* (fig. 15) but I have never seen this parasite in the other two species. In two sets of slides made directly from feces, many individuals of *T. hominis* showed lightly staining coccoid bodies adherent to the external surface (31). In some specimens the cytoplasm also contained these bodies (fig. 16). Whether they represent some phase in the life history of *Sphaerita* or some other organism has not been determined.

Reports of a posterior free flagellum on *T. tenax* (37, 9), or on *T. vaginalis* (37) have never been confirmed on my slides. Sometimes in the living condition, one or more of the anterior flagella become temporarily adherent to the side of the body allowing the tips to protrude beyond the posterior end. Possibly such an occurrence was misinterpreted as showing a free posterior flagellum. When the undulating membrane itself extends beyond the posterior end of the body, as seen in figure 9, its activity could be misinterpreted as that of a free flagellum.

Most authors agree that the posterior flagellum is less than body length in *T. vaginalis* and *T. tenax*, and without a trailing extension such as *T. hominis* has. It is therefore difficult to evaluate the findings of Wagner and Hees (30), who report trichomonads in human blood as well as in the mouth, the intestine and various parts of the female genital apparatus including pathological growths. The morphology of the flagellates from all these sources is essentially the same according to their statements and drawings. In all the latter they show a trailing flagellum, but their photomicrographs of trichomonads from the vagina reveal only the short undulating membrane without a free extension, a condition characteristic of *T. vaginalis*.

*Differential incidence.* Surveys for vaginal, oral and intestinal Protozoa have usually been made

independently of each other, but results of such surveys reveal the greater prevalence of *T. vaginalis* and *T. tenax* in comparison with *T. hominis*. Several surveys in Philadelphia illustrate the point. An incidence of 22.7 percent for *T. vaginalis* was determined by direct examination of 600 gravid women at the Jefferson Medical Hospital (6), while a survey of the mouths of 350 persons, mostly at the same hospital, showed the same percentage of *T. tenax* determined by culture methods (2). On the other hand, a survey of 1060 college freshmen for intestinal Protozoa (36) failed to reveal any trichomonads when a single normal stool per person was examined directly and by stained slides. My own figures for the incidence of *T. hominis* are 24 cases in 2000 persons, an incidence of 1.2 percent. These figures do not suggest that vaginal and oral infections are derived from intestinal sources. However, since the methods in the above mentioned surveys were not the same, figures provided by the examination of all three sites of infection by the three methods of direct examination, prepared slides, and cultures, are more reliable. Such a survey (5) of 200 women at Jefferson Hospital gave an incidence of 23.5 per cent for *T. vaginalis*, 16.5 per cent for *T. tenax* and only 1.5 per cent for *T. hominis*. Only one individual harbored all three species, and only 9, or 4.5 per cent harbored both the oral and vaginal species. These figures do not support the concept of the transferability of trichomonads from the intestine to the mouth and the vagina.

*Cultural differences.* Although all three species may be cultured in the same medium (5), *T. vaginalis* is less adaptable than the others, requiring approximately body temperature, while the other two can be grown at room temperature. *T. hominis* is still more adaptable than *T. tenax*.

It has been reported (1) that after a few days in culture, *T. vaginalis* assumes a form indistinguishable from *T. hominis*, presenting a smaller size, longer undulating membrane and longer axostyle. As previously suggested (25) these changes were probably due to autotomy, the reduction in amount of posterior cytoplasm producing the changes observed. In my experience (4) *T. vaginalis*, after from 6 to 11 months in culture, presented the same distinctive characters that it had at the beginning. All three species retain their specific characters in cultures, as observed by my students and myself, as well as by others (25, 38).

*Animal inoculations.* Elsewhere (35) I have called attention to the occurrence in certain monkeys, cats, dogs and rats of intestinal trichomonads morphologically indistinguishable from *T. hominis* and to reciprocal cross infections which have been made among these various hosts. Inoculations of the other species of man into animals are also of interest. Although the intestines of kittens were readily infected with *T. hominis*, they could not be infected with *T. vaginalis* or *T. tenax* (7). The caeca of baby chicks were successfully infected with both *T. hominis* and *T. tenax* (15) but bacteria-free cultures of *T. vaginalis* failed to infect the caeca of young turkeys and chicks (29).

Experimentation on monkeys is complicated by the natural occurrence of trichomonads in their vaginas (18, 17) and by the presence of at least two species in their intestines (21, 32). Infection of the vaginas of two out of six monkeys inoculated with intestinal trichomonads from the same species of monkey (*M. rhesus*) has been reported (14), but *T. hominis* of man failed to become permanently established in the vaginas of monkeys (16, 22). Both unsuccessful (7) and successful (22, 29) results have been reported in attempts to infect the vagina of monkeys with *T. vaginalis* of man.

Dobell (11) has reiterated his belief that the intestinal and vaginal trichomonads of man belong to the same species. He records that after infecting himself with his strain RT derived from *Macacus rhesus* which had been infected with a strain NT obtained from *M. nemestrinus*, he succeeded in producing a vaginal infection in *M. sinicus* with a culture derived from himself. From this he reaches the conclusion that the intestinal and vaginal trichomonads of man belong to the same species. This conclusion does not seem warranted by this experiment and it disregards all the careful morphological studies and the physiological evidence showing that the vaginal and intestinal forms are distinct.

*Experiments with human hosts.* As would be expected, it is possible to infect the human vagina with cultures of *T. vaginalis* (22, 28), but attempts to transplant *T. hominis* in cultures to the human vagina have failed (22, 28, 26). *T. tenax* in culture also failed to become permanently established in the vaginas of 50 women inoculated, although persisting as long as 432 hours in one host (27). These crucial experiments on human beings, along with all the other evidence, should remove all

doubt as to the separateness of the species in the vagina, the mouth and the intestine, respectively.

*Comments on nomenclature.* There is no question as to the correct name of *Trichomonas vaginalis* Donné, type species of the genus. The species from the mouth has received a number of names including *T. buccalis* (13) and a series of names by Steinberg, including *T. elongata* which has been extensively used. Dobell (12) has recently reminded us of the name *Cercaria tenax* which O. F. Müller first applied to a flagellate in the human mouth in 1773. On the assumption that there is only one such flagellate and that it is a species of *Trichomonas*, Dobell argues for the availability and priority of Müller's species name making *T. tenax* the correct name for the oral form. I believe that this is a justifiable disposition of the problem.

In my experience the intestinal species has a somewhat variable number of anterior flagella, most having four or five, with the latter number being characteristic. The question as to whether this species is separable into different races with three, four and five anterior flagella has not been satisfactorily settled. On the assumption that only one species is commonly found in the intestine and pending further light on the question of flagellar number, it is satisfactory to use for the present the name *T. hominis* (Davaine).

*Tritrichomonas fecalis.* Under this name Cleveland (9) described as a new species a trichomonad found in a tap water culture of human feces. This has been reported from no human host other than the one person from whom it was first obtained. It is therefore an extremely rare parasite of man. Some authors have considered this to be a variant of *T. hominis* but a comparison of figure 18 with figures 11 to 14 will disclose the differences between the two species. *T. fecalis* is larger and coarser than *T. hominis*. There are only three anterior flagella which are very long. The undulating membrane is heavier, usually has more undulations and the double marginal filament is coarser. The costa is more uniform in diameter and there are at least two rows of granules parallel to it, one or more located peripherally and a more deeply situated row. The axostyle is coarser and unusually long. Besides these morphological distinctions, Cleveland determined a number of physiological differences.

There can be no question but that *T. fecalis* is different from *T. hominis*, but, as indicated elsewhere (31) it is very closely similar to a species found in frogs and toads (fig. 19). Cleveland very

properly distinguished *T. fecalis* from *T. angusta* of frogs and toads, but did not compare it with the other species commonly found in amphibian hosts. Not only is *T. fecalis* very closely similar morphologically to the species represented by figure 19, but also in physiological characters. Cleveland readily infected tadpoles and frogs with his species. He found it in a culture of human feces in tap water. I have cultured the comparable form from frogs for several months in sterile pond water to which a little gastric mucin or Loeffler's dried blood serum had been added. I am therefore strongly inclined to the opinion that *T. fecalis* is identical with a species found in frogs and toads (fig. 19). It is this latter species (*T. batrachorum*?) that commonly appears in cultures made from the feces of frogs and toads and not *T. angusta*, which I have never been able to cultivate successfully.

#### SUMMARY

Morphologically, the three species of *Trichomonas* of man are distinct, although *T. vaginalis* and *T. tenax* resemble each other more than either resembles *T. hominis*. *T. vaginalis* is much the larger, the other two being about the same length. *T. vaginalis* is more robust in shape and *T. tenax* is more slender.

*T. vaginalis* and *T. tenax* normally have a single compound blepharoplast which may occasionally appear as two approximately equal elements. In *T. hominis* there are characteristically two blepharoplasts of unequal size, the smaller being ventral in position. The oral and vaginal species have regularly four anterior flagella. *T. hominis* has a variable number, usually four or five, but more characteristically five. One of these is attached to the smaller ventral blepharoplast and the other four to the larger one.

The undulating membrane of *T. tenax* is relatively longer than that of *T. vaginalis*, although typically less than body length in both and without an extension of the marginal filament into a trailing flagellum. *T. hominis* has a full-length undulating membrane with a trailing flagellum. At the margin of the undulating membrane each of the three species has an accessory filament which is more slender than the posterior flagellum.

The costa of *T. vaginalis* and *T. tenax* is very slender and of uniform diameter while that of *T. hominis* is coarser, and thicker in its midregion. Both *T. vaginalis* and *T. tenax* have a well-developed parabasal apparatus, but this is apparently absent in *T. hominis*. The axostyle of *T. hominis*

## DESCRIPTION OF PLATES

All figures have been made with the aid of a camera lucida at a magnification of  $\times 4000$  and reduced about one third in printing. The following abbreviations are used for sources and techniques: C = Culture; C.-A. = Chrom-acetic fixative; fe = feces; Fl = Flemming's fluid; G = Giemsa's stain; H = Heidenhain's hematoxylin; O = Osmic acid fumes; S = Schaudinn's fluid.

## PLATE I

FIGS. 1-6. *T. vaginalis*

FIG. 1. Shows 4 flagella, cytostome, chromatic granules in cytoplasm. S., H.

FIG. 2. Shows parabasal body with bend. C.-A., H.

FIG. 3. Shows extra parabasal body. C.-A., H.

FIG. 4. Large flattened specimen. Note parabasal apparatus, double margin of undulating membrane, axostyle split into fibrils. O., G.

FIG. 5. Shows broad pseudopodium. S., H.

FIG. 6. Detached blob of cytoplasm produced by autotomy. S., H.

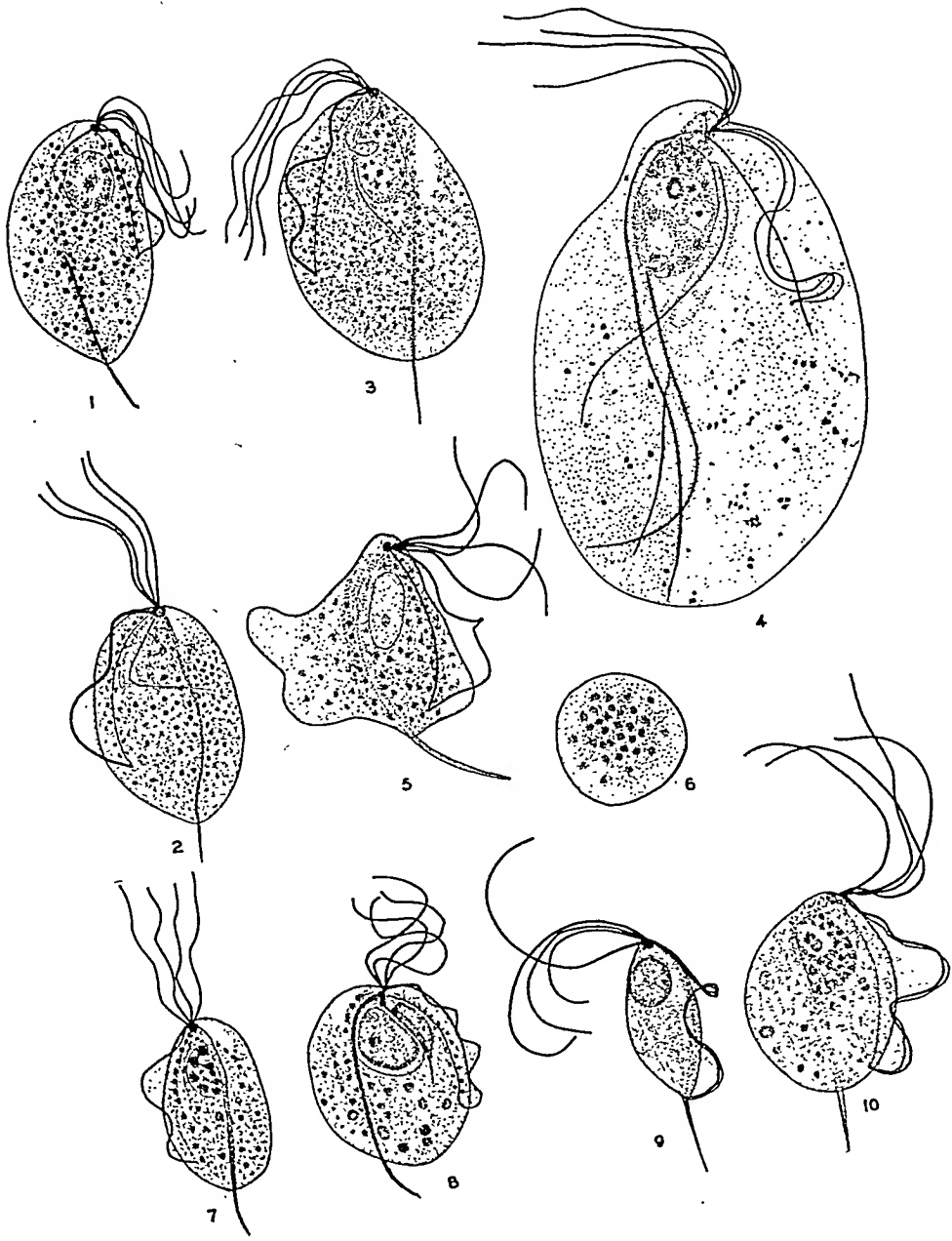
FIGS. 7-10. *T. tenax*, all from cultures.

FIG. 7. Shows paracostal granules, cytostome. S., H.

FIG. 8. Shows parabasal apparatus, paracostal granules and cytostome. Fl., H.

FIG. 9. Deeply stained, undulating membrane full-length. O., G.

FIG. 10. Flattened individual showing double marginal filament. O., G.





## PLATE II

FIGS. 11-17. *T. hominis*.

FIG. 11. Shows 5 ant. flagella, cytostome, paracostal granules, subcostal fibril. fe., S., H.

FIG. 12. Shows 5 ant. flagella, double margin of und. membrane. C., O., G.

FIG. 13. Cytoplasm destroyed; 5 ant. flagella, accessory filament of und. membrane in form of a "fin," 2 blepharoplasts. C., O., G.

FIG. 14. Shows paracostal granules, perinuclear granules, subcostal filament, *Nucleophaga* parasite in nucleus. fe., S., H.

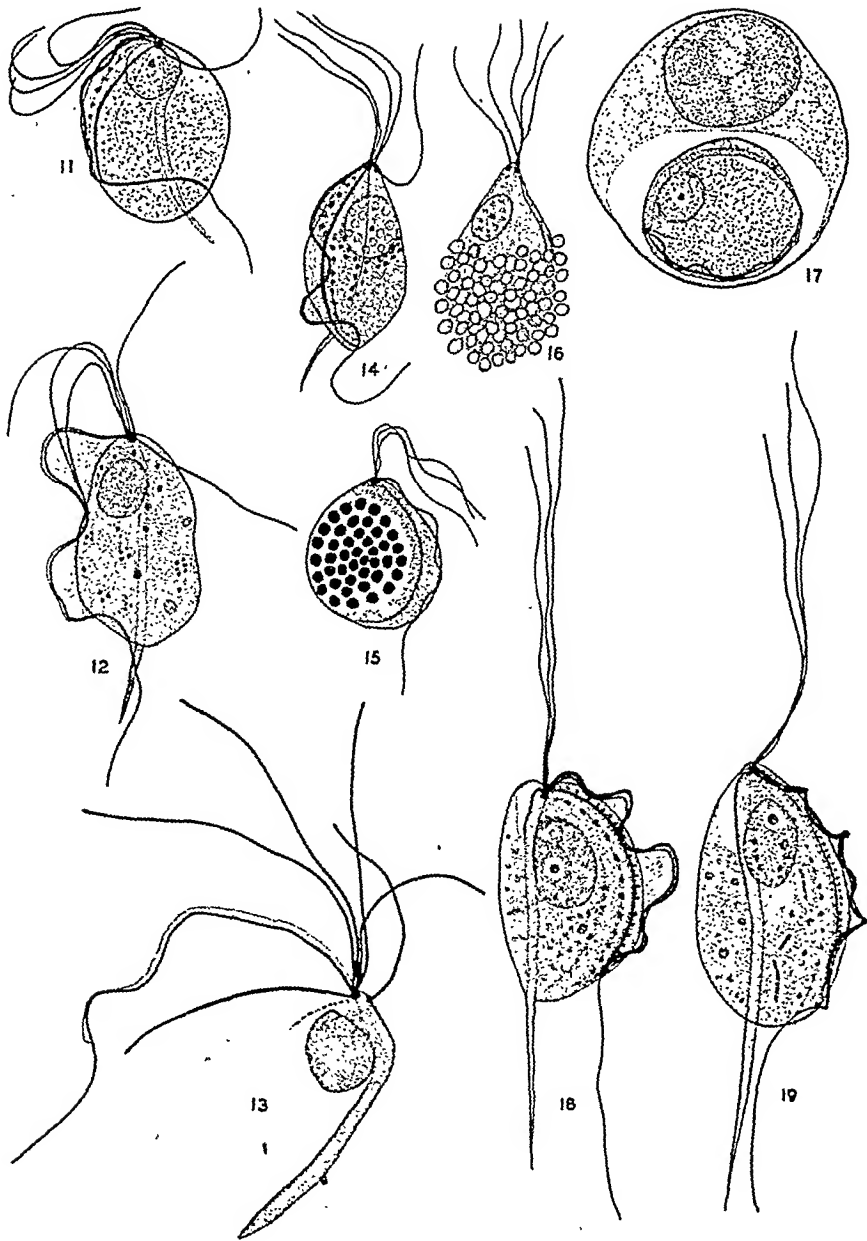
FIG. 15. Shows cytoplasmic parasite, *Sphaerita*. fe., S., H.

FIG. 16. Shows internal and external coccoid bodies. fe., S., H.

FIG. 17. Individual engulfed by a phagocyte. fe., S., H.

FIG. 18. *Tritrichomonas fecalis*, Cleveland slide. C., S., H.

FIG. 19. *Tritrichomonas batrachorum*? from toad. fe., S., H.





## BOOK REVIEWS

*Clinical Laboratory Methods and Diagnosis.* By R. B. H. GRADWOHL, M.D., D.Sc., Third Edition. Illustrated with 726 text figures and 57 color plates. 1943. The C. V. Mosby Company. Two Volumes. Pp. I-XIII, 1-2130. Index, 209 pages.

The third edition of this popular work is really an encyclopaedia of laboratory diagnostic methods, comprising two large volumes containing over two thousand pages, with an index of two hundred pages. Practically all diagnostic laboratory methods of importance are included but it is noted that the laboratory methods employed in the diagnosis of protozoan infections are very briefly considered, the methods described few in number, and the descriptions often sketchy in character. The classification of laboratory methods follows that usually employed in works upon clinical laboratory diagnosis and the amount of space allowed each subject is well divided in accordance with the importance of the subject in diagnosis.

A number of important omissions are noted. For instance, in the description of the malaria plasmodia, no mention is made of *Plasmodium ovale*, while in the discussion of the complement fixation test for amebiasis the original method of Craig is not described, although the results obtained with this method have been better than with other methods which have been developed, when the technique has been strictly followed. Most of the descriptions of the protozoa and helminthes are inadequate. With these exceptions the work is excellent and can be recommended as a reliable guide in clinical laboratory diagnosis. The illustrations are well selected and usually well done while the printing and binding are good. Very few typographical errors were noted by the reviewer.

*Manual of Veterinary Bacteriology.* By RAYMOND A. KELSER, D.V.M., A.M., Ph.D., and HARRY W. SCHOENING, V.M.D., Fourth Edition, pp. I-VII, 1-719. 94 illustrations. The Williams and Wilkins Company, Baltimore, 1943.

This book approaches the status of a classic among bacteriology books. It is very enjoyable reading in its very preciseness of presentation. It is a well-rounded textbook, free of superfluous material, but very suitable for introducing this field to any group particularly interested in comparative pathogenic bacteriology.

That portion of the book on the virus diseases is exemplary. The thoroughness of the sections on

rabies and equine encephalomyelitis attest to the many original contributions of the authors on these subjects. Many rare or little-known virus diseases of animals are described. In addition to the conventional subjects of bacteriology, the allied fields of mycology and protozoology are very adequately presented and the bibliographies are well selected. The close relationship between the fields of human and veterinary bacteriology is repeatedly stressed.

While it is difficult to improve on a book of such wide recognition, its revision does bring many of the active subjects up to date. This is the authoritative textbook on veterinary bacteriology.

*Memoir of Walter Reed. The Yellow Fever Episode.*

By ALBERT E. TRUBY. Brigadier General, United States Army, Retired. Illustrated. Pp. I-XIII, 1-239. Paul B. Hoeber, Inc. New York. 1943.

Many books have been written regarding the discovery of the method of transmission of yellow fever by the mosquito and much misunderstanding has arisen as to the relative parts played in this most important discovery by the members of the United States Army Board and others. The present memoir is written by an officer of the Medical Corps of the Army who was Post Surgeon at Camp Columbia, Cuba, during the investigations, and who can speak with authority regarding the subject.

In his Preface, Brigadier General Truby says: "In recent years, public interest in that great yellow fever episode has developed enormously. Unfortunately, but quite naturally, some distortion of the facts has crept into the picture. Because of that, and the fact that many details of the work and incidents connected with it have never been told, I have repeatedly been urged to write what I know of the personnel, the work they did, their living conditions, and the scene in general." The author has done this and is to be congratulated upon a fine piece of work, and the medical profession and others interested, upon an unbiased account of probably the most important discovery in the transmission of disease which has been made in our times. In a review it is impossible to do justice to this book but it should be read by all interested in the subject as it is well written, authoritative, and as interesting as any novel. General Truby has contributed a most valuable memoir of Walter Reed and those associated with him in the great discovery.



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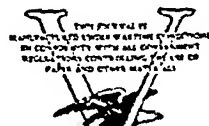
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# PRESENTATION OF THE BAILEY K. ASHFORD AWARD IN TROPICAL MEDICINE OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE

TO DR. NORMAN H. TOPPING

*Past Assistant Surgeon, United States Public Health Service*

*At the Annual Meeting of the Society, November 18, 1943.*

PRESENTATION MADE BY DR. HENRY E. MELENEY

*Mr. President, Ladies and Gentlemen:*

The Bailey K. Ashford Award in Tropical Medicine was established by the American Society of Tropical Medicine through the generosity of Eli Lilly and Company. The award includes a bronze medal, travelling expenses to and from this meeting and a check for \$1000.00. The recipient must be a citizen of the United States, and must be under thirty-five years of age on January 1st of the year of the award. This is the second time that this award has been made. The first award was made in 1941 to Dr. Lloyd E. Rozeboom of Johns Hopkins University for his contributions to medical entomology, and particularly for his demonstration of the importance of *Anopheles bellator* in the transmission of malaria in Trinidad.

As Chairman of the Committee of Award I have the honor to present to you the second recipient, Dr. Norman H. Topping, Past Assistant Surgeon of the United States Public Health Service. Dr. Topping was born on January 12, 1908. After studying at the University of Washington, he transferred to the University of Southern California where he received the degree of Bachelor of Arts in 1933 and that of Doctor of Medicine in 1936. During his medical course he did special work in Bacteriology under Professor John F. Kessel, and was elected to the honorary scientific society of Sigma Xi. He entered the United States Public Health Service in November 1936 and, after assignments in a marine hospital and in the quarantine and coast guard divisions, he joined the staff of

the National Institute of Health where he has remained until the present time. During this period he has been actively engaged in research in the field of the rickettsial diseases. He developed a hyperimmune serum for the treatment of Rocky Mountain spotted fever. He showed by epidemiological and laboratory methods that the eastern and western forms of spotted fever are essentially the same and that both mild and virulent strains occur in both regions. He developed a method for preserving the rickettsiae of spotted fever, typhus, and "Q" fever by rapid freezing and drying *in vacuo*. With Ida Bengtson he demonstrated the value of the complement fixation reaction in the study of rickettsial diseases.

Since the outbreak of the present war Dr. Topping has devoted his time primarily to methods of improving and testing typhus vaccine. His work in developing tests for the protective power of this vaccine has been one of the greatest contributions to the field of rickettsial diseases within the past few years. Unfortunately the secrecy surrounding research of this kind during the war makes it impossible to describe Dr. Topping's work in more detail. During his entire research career Dr. Topping has shown an unusual degree of imagination and originality as well as the unlimited capacity for hard work which is necessary for great accomplishments.

Dr. Topping, on behalf of the American Society of Tropical Medicine I take great pleasure in presenting to you the second Bailey K. Ashford award in Tropical Medicine.



## TYPHUS FEVER

### A NOTE ON THE SEVERITY OF THE DISEASE AMONG UNVACCINATED AND VACCINATED LABORATORY PERSONNEL AT THE NATIONAL INSTITUTE OF HEALTH<sup>1</sup>

NORMAN H. TOPPING

*P. A. Surgeon, U. S. Public Health Service*

*From the Division of Infectious Diseases, National Institute of Health, Public Health Service, Federal Security Agency*

Received for publication January 14, 1944

For some fifteen years cases of typhus fever have been occurring among personnel engaged in investigations of this disease in the Typhus Unit at The National Institute of Health. The early cases occurred before an active immunizing agent was available, while the more recent cases have occurred in vaccinated individuals. Since so little is known concerning the effect of typhus vaccine on a subsequent human infection it is of interest to compare these two series of cases.

During the period of 1929 to 1943, there has been a total of seventeen cases of typhus fever among the personnel, nine of these in unvaccinated and eight in vaccinated individuals. The records of fourteen of these are adequate and can be compared, the records of the other three (two unvaccinated and one vaccinated) are not sufficiently complete and therefore have been excluded. The two unvaccinated cases were typical, moderately severe cases similar in many respects to the others described in detail but the patients remained at home, some miles from the city, and were not seen frequently enough for complete records. The vaccinated case was very mild; although no temperature records were kept it was our impression that the patient was febrile for approximately five days. In most respects this case corresponds to the other mild illnesses among the vaccinated group.

The fourteen cases left for consideration here are evenly divided, seven vaccinated and seven unvaccinated. There were six males and one female in each of the two groups, and the age distribution was quite comparable. The cases all resulted from accidental laboratory infections, although the exact time or circumstances of the effective exposure are unknown.

<sup>1</sup> Read at the Thirty-Ninth Annual Meeting of the American Society of Tropical Medicine at Cincinnati, Ohio, November 16-18, 1943.

#### UNVACCINATED CASES

The onsets were sudden with but few prodromes. Headache, chills, muscle aches, and pains were the chief initial complaints. Upon admission for treatment to the various hospitals in and around Washington the patients were noted as being acutely ill. The fevers were uniformly high and all persisted over two weeks (average 17.1 days). Two of the seven patients (#1 and 3, table 1) became very toxic during the course of their illness and were irrational at times. A definite maculopapular rash was described for all seven unvaccinated patients. It was first noticed in the majority of cases on the fifth or sixth day of the illness, and persisted, either as macules or pigmented spots for at least several days. It was described as occurring mainly on the trunk. These seven patients were confined to bed for about three weeks (average 22.5 days), and their convalescence before returning to work was quite prolonged.

These seven illnesses, judged by the hospital records, were all characteristic of the classical descriptions of typhus fever and there was never a question as to the diagnosis. The clinical impressions were confirmed by a marked rise in the Weil-Felix reaction.

The data for these cases as well as for those that had been vaccinated appear in table 1.

#### VACCINATED CASES

These seven people had received a specific immunizing agent some time before contracting the disease. The first vaccinated individual to develop typhus fever had received only some of the very early yolk sac vaccine, while the other six cases had each received at least one inoculation of a more recent typhus vaccine prepared from yolk sacs. As judged by days of fever, days in bed, and absence from work, as well as the clinical

TABLE 1			
Number of cases of typhoid fever in vaccinated and unvaccinated laboratory personnel			
Vaccination	Sex	Age, years	Duration of exposure, months
Vaccinated	Male	25	1
	Female	25	1
Unvaccinated	Male	25	1
	Female	25	1

[illegible]

## TYPHUS FEVER

R. G. II.	28	M	Early y.s. vaccine	3/21/42	Home	5/29/42	7	4	6	None (no outside physician)	1:20 1:80 1:80 1:80	5/29 6/ 3/42 6/ 3/42 6/11/42 6/16/42	Neg. 1:512 1:1024 1:2048 1:1024	5/29/42 6/ 3/42 6/ 3/42 6/ 8/42 6/11/42 6/16/42
W. B.	50	M	Later y.s. vaccine, 1 cc. dose	8/16/42  8/28	Hosp.	11/23/42	7	8	12	Sinusitis	1:20 1:160 1:640 1:1280 1:160	11/23/42 11/30/42 12/ 7/42 12/12/42 1/30/43	Neg. 1:128 1:512 1:128 1:512	11/23 11/30 12/7 12/12 1/2
L. S.	25	M	Later y.s. vaccine, 1 cc. dose	4/28/42 6/16/42 8/28/42	Home	12/ 3/42	5	6	12	Strep. throat	1:80 1:160 1:160 1:80	12/ 3/42 12/16/42 12/26/42 1/ 2/43	Neg. 1:2048 1:2048 1:4096	12/3 12/16 1/4 1/26
L. F. B.	50	M	Later y.s. vaccine, 1 cc. dose	3/23/42	Home	12/24/42	5	7	7	Pneumonitis	1:160 1:320 1:320 1:160	12/30/42 1/ 4/43 1/18/43 2/15/43	1:4096 1:2048 1:1024	12/30 1/4 1/26
P. P.	22	F	Early y.s. vaccine  Later y.s. vaccine, 1 cc. dose	10/23/42 10/30/42 11/ 6/42 11/15/42	Home	1/15/43	7	10	18	None (no outside physician)	1:20 1:20 1:40 1:40	1/15 1/20 1/26 2/17	1:32 1:256 1:8192	1/15, 1/20 1/26
R. C. B.	37	M	Early y.s. vaccine, 1 cc. dose  Later y.s. vaccine, 1 cc.	11/18/41 11/24/41 12/ 1/42 8/19/42	Home	3/28/43	5	5	6	Grippe	1:640	4/ 5/43	Neg. 1:1024	9/18/42 -4/5

course, this first case (W. W.) was the most severe of those vaccinated.

The onsets for this group were comparatively mild, with headache and fever the outstanding complaint. Only two of the seven patients were hospitalized, the early case of W. W. in 1941 and W. B. in 1942. The latter was a fifty year old male and from past experience a much more serious illness might have been expected. The hospital physician insisted this patient had only a sinusitis, but a few macules were seen at the anterior axillary lines on the evening of the sixth day, which disappeared by the next morning. This finding together with the serological studies indicated that the correct diagnosis was typhus fever. The other five cases were treated in their homes. Two of them were suspected of having typhus fever and were cared for by physicians of the Typhus Unit. No very definite exanthem was ever seen, although on the sixth day in each instance 3-5 macules were observed either in the axilla or on the chest. The other three cases were treated by outside physicians and were diagnosed as "a strep throat," "grippe," and "pneumonitis." They were very mild illnesses and only by the laboratory studies was the diagnosis of typhus fever made.

These seven cases of typhus fever had relatively short febrile illnesses, an average of 6.8 days as compared to 17.1 for the unvaccinated group. Their confinement to bed was but slightly greater (average 7.1 days) than the duration of temperature elevation. In only two cases was convalescence at all prolonged, the early case, and that in a young female. In three of the seven cases the patient returned to full duty in one week from the onset.

Clinically none of these last six cases were considered very ill. Headache and perspiration were the only complaints during the illness. Typhus would not have been considered in four of the cases except for the serological studies. As can be seen from the data presented in the table there was a marked rise in complement-fixing antibody in each case coincident with the illness. The peak titre was reached shortly after defervescence of fever and then gradually declined. It was further noted that the Weil-Felix titres in none of the vaccinated patients reached the heights commonly seen in the unvaccinated ones, in fact three of the seven vaccinated cases failed to develop a Weil-Felix of significant titre.

## DISCUSSION

Marked differences in case severity have been demonstrated in vaccinated and unvaccinated individuals who develop laboratory infection with typhus fever. No attempt has been made to determine which of these cases was infected with an endemic (murine) or which with an epidemic (classical) strain of virus. It might well be that the early cases were infected with endemic virus, since most of the investigations at that time were concerned with the murine strain of typhus fever virus. The more recent cases might well have been infected with the epidemic form since much of our recent work has been with this type of virus. During both periods however, exposure to both forms of virus was undoubtedly present and any attempt at separation now would be untrustworthy.

The second question which naturally arises concerns a loss of virulence in our strains of virus during this fifteen year period. We can only state that when judged by the behavior of these strains in guinea pigs, neither of our two stock strains (Breinl and Wilmington) has made any measurable change during this period. The incubation period and the duration of fever in the guinea pigs is identical to those of ten years ago. Exposure in the recent cases was mainly to typhus virus which had been cultivated in fertile eggs. It is possible that with adaptation to the yolk-sac the virus may lose some of its virulence for humans.<sup>2</sup>

It is of considerable practical importance that a persistently negative Weil-Felix reaction may not be a reliable test in excluding typhus fever in vaccinated individuals. If we had relied entirely upon a positive Weil-Felix test for laboratory confirmation we would have missed several of our cases.

In this connection it might be well to recall Munter's observations in 1929. He inoculated rabbits with typhus virus and after a suitable time they developed agglutinins for *Proteus* OX 19. After several months, when the Weil-Felix had returned to the normal level, the rabbits were re-inoculated, some with Rocky Mountain Spotted Fever and some with typhus virus. Those receiv-

<sup>2</sup> Since this was written, we have had a case of typhus fever in an unvaccinated individual, a casual visitor. His course was very similar to that of S. E. S. in table 1. In this case the incubation period was fourteen days following exposure to yolk-sac virus of the Breinl strain of epidemic typhus virus.

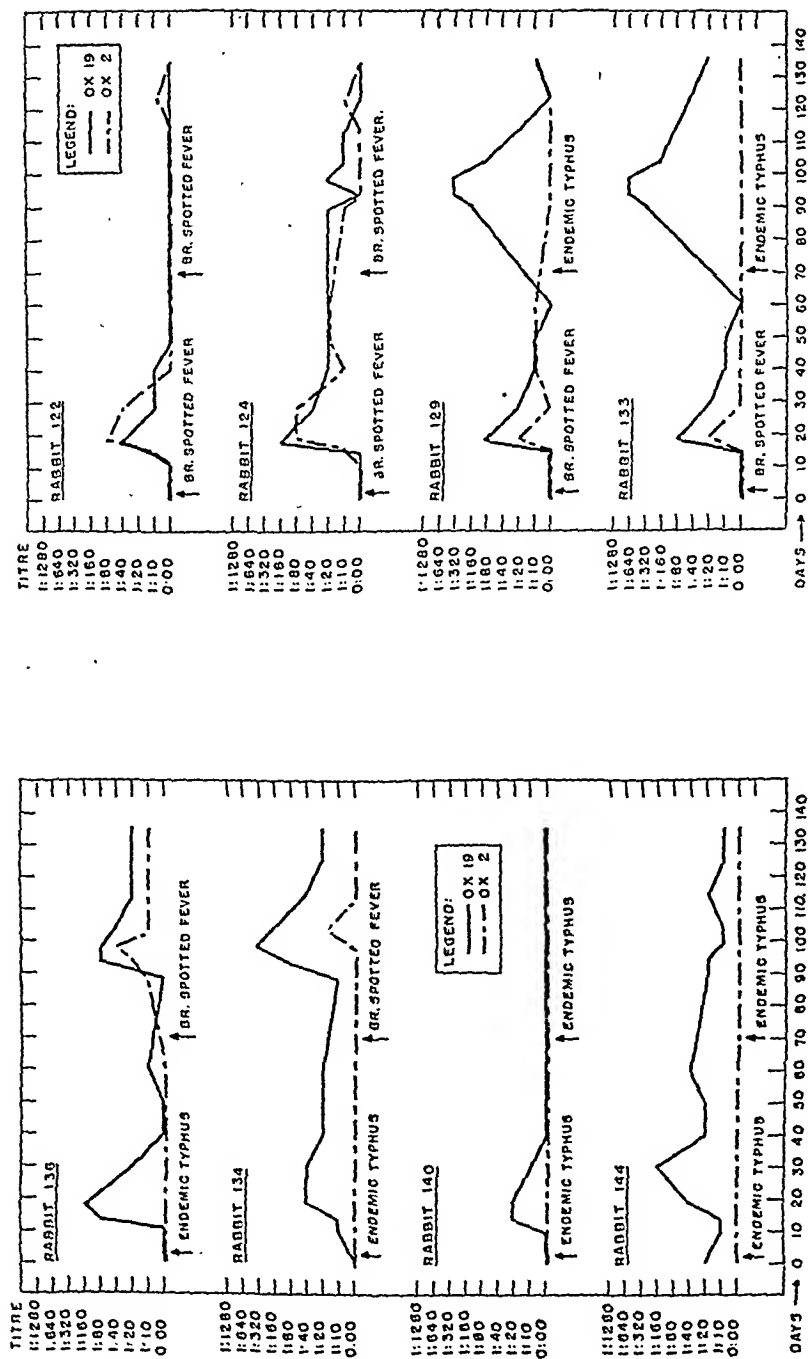


CHART 1



ing the heterologous material again developed a positive Weil-Felix but those receiving the homologous strain failed to develop a rise in agglutinins for *Proteus* OX 19. Several years ago Munter's observations were confirmed in this laboratory. Chart #1 is included to illustrate graphically the results. It is possible that potent typhus vaccine may produce sufficient immunity in humans that they, when subsequently infected, fail to produce consistently agglutinins for *Proteus* OX 19. This might be of importance in morbidity studies in groups of vaccinated and unvaccinated people, especially so if the Weil-Felix test is the only one being used as confirmation of the clinical diagnosis.

With the exception of the first vaccinated case of typhus the symptoms in the vaccinated group were very mild and quite atypical. This modification in the clinical course of the illness may offer difficulties in the diagnosis, and should be kept in mind in areas of typhus fever where widespread vaccinations have been accomplished.

Mooser has indicated that the amount of functioning virus and the number of infected lice is

proportional to the severity of the illness. We attempted isolation of virus from two of the unvaccinated cases (S. E. S. and H. C. T.). A single sample of blood was withdrawn about the fifth day of illness and inoculated into guinea pigs. From H. C. T. this resulted in the isolation of a typical endemic strain of typhus fever. Daily attempts at isolation of virus were made on two of the vaccinated cases (W. B. and P. P.), and on a third case (R. G. H.) three attempts were made. Whole blood was inoculated into fertile eggs as well as intraperitoneally into guinea pigs, but we were unsuccessful in isolating the etiological agent. Our experience suggests that there may be quantitative differences in the amount of circulating virus between cases of typhus fever in vaccinated and unvaccinated individuals. If typhus vaccine will modify cases in the field as it apparently modifies laboratory infections, it might interrupt the epidemiological chain of louse-man-loose and control epidemics of typhus fever even though not entirely preventing the disease.

# THE AMERICAN SOCIETY OF TROPICAL MEDICINE<sup>1</sup>

## A BRIEF BIOGRAPHICAL SKETCH

ERNEST CARROLL FAUST

*From the Department of Tropical Medicine, the Tulane University of Louisiana, New Orleans, La.*

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### PROLOGUE

As the writer has perused the records of the Society, including those of the annual business and scientific sessions, the numerous council meetings, the secretary's reports and Doctor Swan's brief history of the first decade of its existence, he has come to regard the organization as a living entity, with desires and ambitions, successes and failures. Moreover, not all of the events of any being's existence are a matter of record, and some of the unrecorded occurrences have even more warmth and personality than the recorded ones. Such has been the case with our Society. The more important incidents in the first forty years of the life of the Society form the basis for this brief biographical sketch.

### FORMATION AND EARLY YEARS OF THE SOCIETY

According to the first entry in the Minute Book of the Society "a meeting was held at 1319 Spruce St., March 9, 1903 for the formation of a Society in Philadelphia for the Study of Tropical Diseases." For several months previously the matter had been under consideration, as indicated in the following two letters written by Doctor Thomas H. Fenton. The first was dated January 29, 1903 and was addressed to Dr. W. W. Keen; the second was dated February 28, 1903 and was addressed to a number of practicing physicians in Philadelphia.

Dear Dr. Keen,

As is well known, there are many areas within the United States proper which are subtropical, and the new possessions of our country are almost or wholly tropical. This would seem to make it necessary that the profession should give closer attention, perhaps, to what are considered tropical diseases. It will certainly be admitted that in a centre of medical education like Philadelphia there should be opportunity afforded for this kind of study. This view is confirmed by the

recent establishment in this city of a course of lectures on "Tropical Diseases". I have thought for a long time that the existence of a society, at the meetings of which these topics would be considered, would perhaps act as a stimulus to the promotion of these studies, and favor the development and increase of our knowledge in this particular direction. All to whom I have spoken are heartily in accord with the proposal, and seem, furthermore, willing to join such an organization. The society need not be a large one nor involve, by frequent meetings, too much tax upon the time of a busy practitioner. If you incline favorably to the views expressed, I shall be glad to have you meet a few physicians at my house on Monday evening, February 2nd, at 8:15, for the purpose of discussing the expediency of forming such a society.

Very truly yours,

THOMAS H. FENTON

Dear Doctor:

At an informal meeting held Feb. 2nd, the question of forming in this city a society for the study of Tropical Diseases was favorably considered. The consensus of opinion of those present and of a number who had sent letters in response to the call, was, that the time is ripe for the formation of such a society in Phila., somewhat on the following lines. The membership should be somewhat restricted in numbers and should include a list of honorary and corresponding members, the dues should be merely nominal and the number of meetings confined to three or four yearly. If you incline favorably to this view, the undersigned Committee will be glad to have you attend a meeting for the further consideration of the subject, on Monday, March 9th, at 8:30, P.M., at 1319 Spruce St.

THOMAS H. FENTON

*Sec.*

WHARTON SINKLER.

JUDSON DALAND.

JOSEPH MCFARLAND.

JAMES C. WILSON.

JAMES M. ANDERS.

*Committee*

The years immediately preceding the birth of the Society had been very important to American physicians in the field of tropical medicine. As never before the problems of disease in warm

<sup>1</sup> Read at the Thirty-Ninth Annual Meeting of the American Society of Tropical Medicine, Cincinnati, Ohio, November 16-18, 1943.

climates had been thrust upon their consciousness by the American occupation of the Philippines and Puerto Rico and the undertaking of the Panama Canal. Cholera and plague had invaded the Western Hemisphere. Yellow fever was still unconquered, even though Walter Reed and his colleagues had provided the solution and Gorgas had started his successful sanitary campaigns to eradicate the disease from urban centers. Malaria, hookworm disease and deficiency diseases were serious public health problems in the Southern United States as they were in the American Tropics.

The charter group of the Philadelphia Society consisted of well known clinicians belonging to the College of Physicians of Philadelphia, but they were not experienced in the field of tropical medicine.

Thirty-eight doctors indicated their intention of joining the new Society but only twenty-one became active members in 1903 and only twenty-eight signed the charter. They included the following: *internists* (Roland G. Curtin, Hobart A. Hare, James M. Anders, James C. Wilson, B. Franklin Stahl, Judson Daland, John H. Musser, Alfred Stengel, J. Chalmers Da Costa, John M. Swan); *pathologists* (Joseph McFarland, W. M. L. Coplin); *neurologists and neuropsychiatrists* (T. H. Weisenberg, D. J. McCarthy, F. X. Dercum, J. K. Mitchell); *dermatologists* (Henry W. Stelwagon, John V. Shoemaker); a *surgeon* (W. W. Keen); *ophthalmologists* (Thomas H. Fenton, George E. de Schweinitz, S. D. Risley); *ear, nose and throat specialists* (E. B. Gleason, B. Alexander Randall); a *urologist* (Orville Horwitz); a *therapist* (S. Solis Cohen), and *general practitioners* (Wharton Sinkler, Samuel G. Dixon). The majority of this group held outstanding professorial positions in the faculties of the University of Pennsylvania, the Jefferson Medical College and the Medico-Chirurgical College which later became the Post-Graduate Medical School of the University of Pennsylvania.

At the organization meeting by-laws were formulated and the following officers and councilors were elected: President, Thomas H. Fenton; Vice-Presidents, James C. Wilson and James M. Anders; Secretary, Joseph McFarland; Assistant Secretary, no nomination (position soon thereafter filled by election of John M. Swan); Treasurer, E. B. Gleason; Councilors, John V. Shoemaker, Roland C. Curtin, Orville Horwitz, Judson Daland, Hobart A. Hare. The name selected for

the organization was "Society of Tropical Medicine of Philadelphia," but at the first council meeting held March 20, 1903 a motion was carried to change the name to the "American Society of Tropical Medicine" "to conform to the national idea," and this change was incorporated in the by-laws. These governing articles provided for three groups of members, active, corresponding and honorary. Only physicians were eligible for election to the first two groups, while an honorary member was defined as "a scientist who has made eminent contributions to tropical medicine."

On May 25, 1903 a charter was adopted which was to incorporate the AMERICAN SOCIETY OF TROPICAL MEDICINE in the County of Philadelphia, State of Pennsylvania. (This charter did not actually become effective until some time between February 18 and March 21, 1904, following its signing by the charter membership.) The Society operated under this legal instrument until 1933, when a new Constitution and By-Laws were adopted.

The first honorary members, elected on December 7, 1903, were as follows: Brig.-General William H. Forwood (M.C., U.S.A.), Rear-Admiral P. M. Rixey (M.C., U.S.N.), M. J. Rosenau, Charles W. Stiles, W. C. Gorgas, James Carroll, D. E. Salmon, Aristides Agramonte, John Guiteras, Patrick Manson, George H. Nuttall, A. Laveran, Robert Koch, Major George Lamb, A. Calmette, William Murrell, C. F. Martin, William H. Welch, George M. Sternberg, Ralph Stockton, Charles D. F. Philips and Frederick Montizambert.

Meanwhile the Society planned to hold two or three scientific meetings a year in Philadelphia in addition to the Annual Meeting, in order that medical students as well as physicians might become acquainted with the special problems and advances in tropical medicine. The first of these public sessions was held at the University of Pennsylvania on January 9, 1904 and was addressed by James Carroll, Surgeon, U. S. Army, on "The Etiology of Yellow Fever."

On February 18, 1904 nine new active members were elected, including three who resided outside the Philadelphia area.

The First Annual Meeting of the Society was held in Lower Hall, College of Physicians of Philadelphia on March 21, 1904. It consisted of an exhibit of pathological material on anesthetic leprosy, amebic and bacillary dysentery, liver abscess, duodenum "with uncinariae *in situ*," *Leishmania donovani* in blood films sent by Sir

Patrick Manson, and *Hymenolepis nana*, and was followed by a private business meeting. At this session the Secretary reported that there were 30 active members, six corresponding members and 22 honorary members.

At the Council Meeting on December 4, 1904 it was unanimously voted "to recommend to the Society that some action be taken upon the suggestion of Sir Patrick Manson, that it would be wise to endeavor to secure public school training in the hygienic prophylaxis of tropical diseases in

conjunction with large medical gatherings. Beginning with the 27th Annual Meeting (1931) the yearly sessions have year by year been held in association with the Southern Medical Association.

#### MEMBERSHIP

The original By-Laws called for election to active and to corresponding membership of physicians interested in tropical medicine, and to honorary membership of eminent scientists whose contributions included tropical diseases. These three

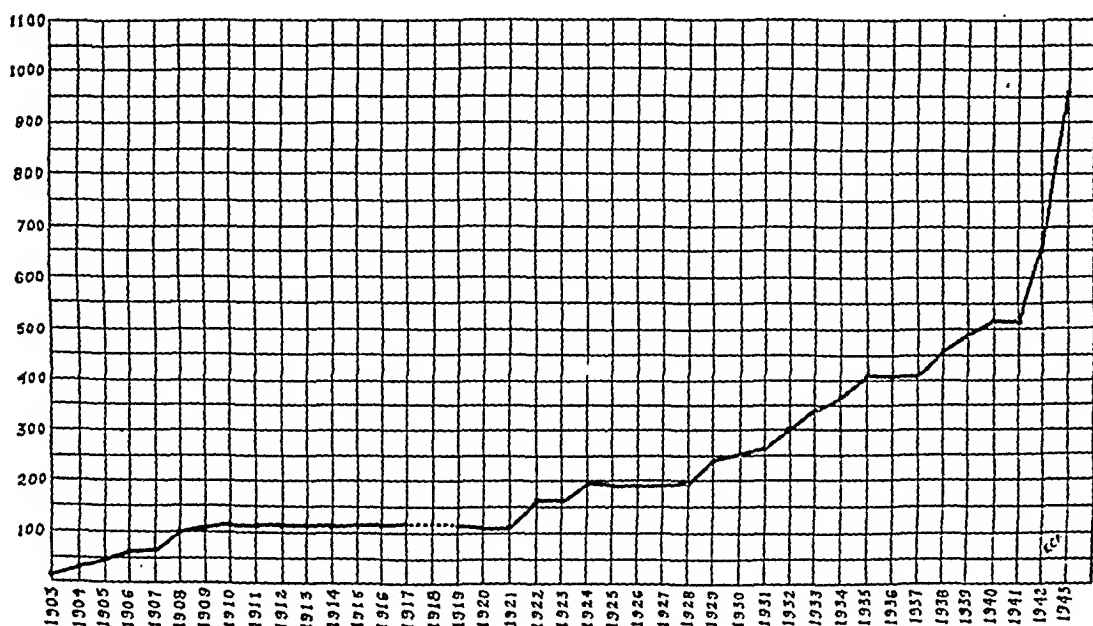


FIG. 1. Active membership curve of the American Society of Tropical Medicine. The number of members in good standing is indicated on the ordinate and the years on the abscissa. The broken line indicates the year (1918) when no meeting was held on account of the influenza epidemic.

Hawaii, Porto Rico and the Philippine Islands." (Adopted at the Second Annual Meeting, March 24, 1905). At this same council session it was urged that lists of nominees for active membership be secured from outside Philadelphia. This effort was fruitful, since the Society elected at the Second Annual meeting 26 active members, of whom only four were Philadelphians.

The Third Annual Meeting convened in Philadelphia on March 21, 1906. Although public scientific sessions were subsequently held in Philadelphia under the auspices of the Society, all annual meetings following the third were convened in other cities, at times separately, but for the most part and with increasing consistency in

categories were maintained through 1928, when the corresponding membership list was abolished. Corresponding and honorary membership lists included both American and foreign workers in the field, many of whom were the most distinguished contributors of their time to tropical medicine. At the Fifth Annual Meeting (March 28, 1908), paragraphs 2 and 3 of Article I of the By-Laws were amended so that medical scientists might be admitted to active and corresponding membership. This step was taken because at a previous meeting a distinguished medical zoologist not holding an M.D. degree had by mistake been elected to active membership. To rectify this error *ad interim* this member was transferred to honorary

membership. At the following (Sixth) Annual Meeting (April 10, 1909) the first "scientist" was technically elected to active membership, namely

dency of the Society in 1909. In 1939, at the Thirty-fifth Annual Meeting, the Society adopted amendments to the Constitution (1933), creating

TABLE 1  
*Membership of the American Society of Tropical Medicine*

YEAR	ACTIVE	CORRESPONDING	HONORARY	EMERITUS	LIFE	REMARKS
1903	21	0	22			
1904	30	6	34			
1905	50	6	36			
1906	61	3	40			
1907	66	17	41			
1908	99	17	42			
1909	107	18	42			
1910	131	17	42			
1911	120	17	40			
1912	120	17	37			
1913	121	17	37			
1914	113	17	37			
1915	112	17	35			
1916	117	17	33			
1917	119	16	30			
1918	...	..	..			No meeting; influenza year.
1919	123	17	35			
1920	108	19	29			
1921	107	11	27			
1922	163	18	17			
1923	163	18	17			
1924	191	22	17			
1925	181	16	17			
1926	180	17	15			
1927	190	21	15			
1928	195	22	15			Corresponding Memberships Abolished
1929	237		15			
1930	252		15			
1931	261		14			
1932	300		12			
1933	340		18			New Constitution
1934	366		15			
1935	412		21			
1936	403		16			
1937	410		19			
1938	458		22			
1939	488		22			Emeritus and Life Memberships Established
1940	520		21			Corresponding Memberships Reestablished
1941	516		20	3		
1942	650		20	4		
1943	952		20	4		

Miss Clara S. Ludlow, Ph.D. In passing, it is interesting to note that Dr. W. C. Gorgas was requested to resign from honorary membership in order that he might be made an active member and thus become eligible for election to the Presi-

the categories of Life and Emeritus members, and in 1940, at the Thirty-sixth Annual Meeting, an additional Constitutional Amendment reestablished corresponding membership for persons resident outside the Continental United States.

By 1911 the active membership list had reached 120. Many of the original group in Philadelphia resigned as their interest gradually waned, a few members had died, while several were dropped after non-payment of dues for five years. An appreciable increase in active membership attended the publication of the American Journal of Tropical Medicine in 1921. Since that period the growth in membership has been substantial and during the period since 1941 it has increased with unusual rapidity. As of November 10, 1943 there were 952 active members in good standing of whom 316 had joined since the Annual Meeting in November 1942. 399 of the total were personnel of the armed forces of the United States. The emeritus group included 4 and the honorary membership 20 (see fig. 1, table 1).

#### PUBLICATIONS

From the first year of the Society's existence there was evidence of a desire for the dissemination of printed information in its special field. Not only the lectures delivered at the Annual Meetings and at public scientific meetings but papers sent in by corresponding members provided copy for publication. During the first decade of its history these manuscripts were published in a wide range of medical journals, including New York Medical Journal, American Medicine, Journal of the American Medical Association, Journal of Infectious Diseases, Boston Medical and Surgical Journal, American Journal of Medical Sciences, New Orleans Medical and Surgical Journal, Medical Record, Southern Medical Journal, Archives of Internal Medicine and Journal of Experimental Medicine. Reprints of these papers were bound together and issued to the membership and to exchange correspondents, beginning with volume I (1904-1905) and continuing through volume VI (1912). Thereafter the scientific papers were published *de novo* (volumes VIII and IX, 1913 and 1914) by the Tulane University Press; volumes X, XI and XII, 1916, 1917, 1918, by the New Orleans Medical and Surgical Journal and from 1920 to the present time in the Society's own official organ, The American Journal of Tropical Medicine. During the year 1915 these papers, without volume designation, appeared in the American Journal of Tropical Diseases and Preventive Medicine (New Orleans).

The American Journal of Tropical Medicine, the official Society publication, is so well known to all of the membership and friends of the Society

that only brief mention need be made of it and of its editors. Its inception was due primarily to Doctor Henry J. Nichols, who negotiated a favorable contract for its publication with Williams & Wilkins in 1920. Beginning in January, 1921, and bimonthly since that time the Journal has built up an increasing number of member and non-member subscribers. Doctor Nichols nurtured this offspring through its first six years until his sudden death in 1927, since which time it has developed to maturity under the able editorship of Doctor Charles F. Craig.

#### PATHOLOGICAL COLLECTION AND LIBRARY

During the first decade of its existence the Society actively cultivated a museum of tropical diseases and a library. These no doubt created considerable tangible pride on the part of the membership but they also provided a basis for acquiring first-hand and authoritative information on parasitic and tropical medicine. Many of the honorary members made generous contributions to both of these assets and the collected volumes of the Society's Transactions made advantageous exchanges possible. During the earlier years of its life the Minute Book of the Society faithfully recorded the receipt of all these contributions. The books and reprints were deposited in the Army Medical Museum and later placed in the Allen J. Smith collection of the Army Medical Library. The writer has been unable to learn where the pathological material and the charter, the original legal instrument of the Society, are located.

#### OFFICERS

Each year, with the publication of the Minutes of the Annual Meeting, a list of the past presidents of the Society has been incorporated. No such record of the other officers has been included. Since the life and growth of the Society has in considerable measure been due to the untiring efforts of the Secretaries, it seems desirable to incorporate in this sketch a list of these devoted workers, together with the periods when they served:

Joseph McFarland (1903-1906)  
 John M. Swan (1907-1918)  
 Sidney K. Simon (1919-1921)  
 Brayton Howard Ransom (1922-1924)  
 Benjamin Schwartz (1925-1928, 1930-1931)  
 E. Peterson (1929)  
 Henry E. Meleney (1932-1934)

Alfred C. Reed (1935)

Paul Hudson (1936-1937)

E. Harold Hinman (1938-1942)

Joseph S. D'Antoni (1943-)

For a period of a decade or more preceding the adoption of the new Constitution in 1933 it had become the precedent that a member elected as councillor would year by year be elevated one step nearer the presidency and would attain the chair in eight years, even though he might not have attended any of the annual meetings during the intervening years. This dangerous custom, together with the fact that the Society was not complying with the requirement of the Charter that the annual corporation meetings be held in Philadelphia, led to the adoption of the new Constitution.

#### ANNUAL MEETINGS

As previously stated, the first three annual meetings (1904, 1905, 1906) were held in Philadelphia, and thereafter the meetings took place in other cities. The Fourth Annual Meeting (1907) was held in New York; the Fifth (1908), at the Johns Hopkins University in Baltimore; the Sixth (1909), at the U. S. Naval Medical School in Washington, D. C.; the Seventh (1910), at the St. Louis University Medical School in St. Louis; the Eighth (1911), at Tulane University in New Orleans; the Ninth (1912), in Atlantic City; the Tenth (1913), with the Congress of American Physicians and Surgeons in Washington, D. C.; the Eleventh (1914), at Harvard Medical School in Boston; the Twelfth (1915), on the ground of the Pan-American International Exposition in San Francisco; the Thirteenth (1916), in Washington, D. C.; the Fourteenth (1917), in New York; (the 1918 meeting, which was scheduled in Asheville, N. C. with the Southern Medical Association, was called off because of the epidemic of influenza); the Fifteenth (1919), in Atlantic City; the Sixteenth, (1920), in New Orleans; the Seventeenth (1921), in Hot Springs, Ark.; the Eighteenth (1922), in Washington, D. C.; the Nineteenth (1923), in San Francisco; the Twentieth (1924), in Chicago; the Twenty-First (1925), in Washington, D. C.; the Twenty-Second (1926), in Washington, D. C.; the Twenty-Third (1927), in Boston; the Twenty-Fourth (1928), in Washington, D. C.; the Twenty-Fifth (1929), with the Southern Medical Association in Miami, Fla.; the Twenty-Sixth (1930), with the American Association for the Advancement of Science in

Cleveland; the Twenty-Seventh (1931), with the Southern Medical Association, in New Orleans; the Twenty-eighth (1932), with the S. M. A. in Birmingham; the Twenty-Ninth (1933), with the S. M. A., in Richmond; the Thirtieth (1934), with the S. M. A., in San Antonio; the Thirty-First (1935), with the S. M. A. in St. Louis; the Thirty-Second (1936), with the S. M. A., in Baltimore; the Thirty-Third (1937), with the S. M. A., in New Orleans; the Thirty-Fourth (1938), with the S. M. A., in Oklahoma City; the Thirty-Fifth (1939), with the S. M. A., in Memphis; the Thirty-Sixth (1940), with the S. M. A., in Louisville; the Thirty-Seventh (1941), with the S. M. A., in St. Louis; the Thirty-Eighth (1942), with the S. M. A., in Richmond; and the Thirty-Ninth (1943), with the S. M. A., in Cincinnati.

#### LECTURESHIPS

During the early years of the Society in Philadelphia attempts were successful in bringing to the membership and guests distinguished lecturers on tropical diseases. However, it was not until 1936 that the first lectureship was actually established. Beginning in that year and annually since then the Charles Franklin Craig Lecture has been delivered by authorities in special fields of tropical medicine.

#### AWARDS AND MEDALS

*Walter Reed Medal.*—In 1933 the Council requested that the Society appoint a special committee to investigate the "establishment of a medal to be awarded periodically." The following year at the Thirtieth Annual Meeting this Committee on the Medal recommended that the medal be designated as the "Walter Reed Medal" and that it "be awarded in recognition of meritorious achievement in tropical medicine by an individual or an institution." (The conditions of award of the Walter Reed Medal were published in the American Journal of Tropical Medicine, vol. 19, pp. 94-96, 1939). This medal (cast in bronze) was first awarded at the Thirty-Second Annual Meeting (1936). One medal was presented to the widow of Walter Reed and one was awarded to the Rockefeller Foundation for its study and control of yellow fever. Additional awards have been made as follows: In 1939, to Dr. William B. Castle, of Harvard University; in 1940, to Dr. Herbert C. Clark, of the Gorgas Memorial Laboratory; in 1942, to the United States of Brazil "for outstanding work in the eradication of *Anopheles*

*gambiae* from Brazil," and to Dr. Carlos J. Findlay (posthumously).

*Bailey K. Ashford Award and Medal.*—In 1940 the Council accepted the generous offer of Eli Lilly and Co. to award a sum of One Thousand Dollars, together with a bronze medal, to an outstanding worker not over thirty-five years of age, the award to be designated as the Bailey K. Ashford Award. This honor was bestowed for the first time in 1941, on Dr. Lloyd E. Rozeboom, of the Johns Hopkins University, while in 1943 Dr. Norman Topping, of the National Institute of Health, U. S. P. H. S., was designated as the awardee.

#### SEAL

In 1936 the Council adopted an Official Seal for the Society. Around the rim of the seal is the caption "American Society of Tropical Medicine." Within the inner circle in a tropical setting is a Roman goddess of health on a stone seat, extending a lamp of knowledge to a venomous serpent, the symbol of healing. In the lower left quadrant, engraved on the seat and its pediment, is the motto "salus in tropicis."

#### THE SOCIETY AND TROPICAL MEDICINE

The members of the organizing group in Philadelphia were not acquainted with the diseases of warm climates but were impelled by a desire to learn and to have their medical students become familiar with these diseases. They were fortunate in selecting as honorary members men like Patrick Manson and William C. Gorgas, who inspired them and directed their energies. Soon afterward American practitioners and investigators in the malarious and hookworm-infested Southern States, in Cuba, the Panama Canal Zone, the Philippines,

China and Africa became active and corresponding members and added both experience and interest to the Society. The membership were in no small measure responsible for the formation of the original National Malaria Committee, while the epidemiological observations and clinical studies of several members compelled recognition of the importance of hookworm disease in the Southern United States, leading to the organization of the Rockefeller Hookworm Commission.

The Journal of the Society has year by year since its founding in 1920 disseminated an increasing amount of valuable information throughout the world.

The American Society of Tropical Medicine has always been intensely interested in formal training in tropical medicine both for post-graduate physicians and undergraduate medical students. In its early days it recorded its approval of the Post-Graduate Courses in Tropical Medicine at the University of Pennsylvania and in later years at business sessions considered recommendations for sponsorship of Schools of Tropical Medicine at the University of California and at Tulane University. Since 1939 there have been two Committees on Education in Tropical Medicine which have stimulated interest in the subject on the part of the Association of American Medical Colleges and the National Research Council.

Today, as the Society looks into its forty-first year of activity, with an active membership of 952, it speaks for American medicine throughout the warm climates of the world. Its present and future responsibilities will be increasingly heavier. It can not be content with the aim of the founding fathers "to study tropical diseases," but must put knowledge into action by bringing these diseases one by one under control.





# SUSCEPTIBILITY OF MARMOSETS TO DIFFERENT STRAINS OF YELLOW FEVER VIRUS<sup>1</sup>

H. W. LAEMMERT, JR., M.D.

*From the Laboratory of the Yellow Fever Research Service, Rio de Janeiro, Brazil*

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## INTRODUCTION

While Primates in general are susceptible to infection with yellow fever virus, marmosets and tamarins, which belong to the family Callitrichidae, were considered to be less susceptible than most other Primates.

Stokes, Bauer, and Hudson (1), in their classic study on yellow fever in West Africa, included Brazilian marmosets among the animals used for the isolation of the causative agent. The blood of each of two patients suffering from yellow fever was inoculated into a marmoset and a rhesus monkey. In one instance, the virus was isolated in the monkey but not in the marmoset; in the other, the monkey survived and became immune, while the marmoset died of unknown cause. Davis (2) was able to carry yellow fever virus through at least four passages in both the marmoset (*Callithrix albicollis*) and the tamarin (*Leontocebus ursulus*). Lloyd and Penna (3) reported that intracerebral inoculation of the French neurotropic strain produced a uniformly fatal encephalitis in *Callithrix* monkeys; similar inoculation in *Leontocebus ursulus* usually was followed only by a febrile reaction, and only an occasional animal developed signs of central nervous system involvement and died.

Since in many parts of Brazil in which jungle yellow fever occurs marmosets are very numerous and may conceivably play a role in the epidemiology of the disease, the susceptibility of these animals to different strains, especially jungle strains, of yellow fever virus was studied.

## MATERIAL AND METHODS

*Animals.*—As there is still some confusion concerning the terminology of marmosets, a short description of the species used will be given.

<sup>1</sup> The work on which these observations are based was carried out under the auspices of the Serviço de Estudos e Pesquisas sobre a Febre Amarela (Yellow Fever Research Service), which is maintained jointly by the Ministry of Education and Health of Brazil and the International Health Division of The Rockefeller Foundation.

The marmosets *Callithrix leucocephala* (E. Geoff.) were captured by our field unit in two localities in the State of Espírito Santo (Morro de Argolas, Vila Velha, City of Vitória, and Pau Gigante, Distrito da Sede). These animals are of medium size; adults have a total length of 500 to 550 mm. They are white or whitish on the front of the head and throat, have black preauricular hair tufts, and black ears. The body has a mottled appearance due to the coloration of the individual hairs, which are black at the base, then ocher with a subterminal black bar and white tips. The hands and feet are blackish-brown and the tail is ringed, black and white.

The marmosets *Callithrix jacchus* (L.) came from various parts of Brazil. A few were captured by our field unit in the Tijuca woods, Federal District. All the others were bought at the local market and it was stated that they came from the States of Rio de Janeiro, Sergipe, Pernambuco, and Baía. According to general opinion *C. albicollis* (Spix) is a synonym of *C. jacchus* (L.) and therefore the marmosets which were used by Davis and by Lloyd and Penna belong to this species. This species is of medium size; the total length of adults is 500 to 550 mm. Mature animals have a brownish-black head and nape, a brownish-black neck which is often whitish, a whitish spot on the forehead, and white or whitish preauricular tufts often tipped with black. The lower back is barred with ocher, black, and white, due to the individual hairs which have this coloration. The hind limbs, hands, and feet are black, washed with yellowish-white. The tail is banded black and white. Undeveloped animals have a general grayish coloration, with gray preauricular tufts which gradually turn white with increasing age.

The lion marmosets *Leontocebus rosalia* (L.) were all purchased in the local market and presumably came from the State of Rio de Janeiro. They are somewhat larger than the two preceding species. The total length of adults is around 600 mm. General color of head, body, and limbs is a golden yellow, darker (intermixed with blackish hair) on the head, limbs, and distal part of tail which is

bushy. The tufts of the inside of the ear are brownish-black. The face and inside of the hands and feet are raisin colored.

*Methods of infection.*—All marmosets were inoculated subcutaneously with infectious monkey serum; desiccated material was rehydrated to the original volume with distilled water and diluted to the desired concentration with 10 per cent normal monkey serum-saline. At first the volume of the inoculum was 0.5 ml., but this was soon reduced to 0.1 ml. and finally to 0.06 ml. The virus preparations used were always titrated in albino Swiss mice of eighteen to twenty-eight days of age. Serial tenfold dilutions of the rehydrated virus were made in serum-saline and each dilution was inoculated intracerebrally in 0.03 ml. amounts into a group of six or twelve mice. All surviving mice were reinoculated intracerebrally after thirty days with approximately  $5 \times 10^2$  MLD of French neurotropic virus. Those which survived this challenge dose were considered to have received virus at the first inoculation. The titers were calculated by the method of Reed and Muench (4) on the basis of a combined 50 per cent mortality and immunity, and the total number of mouse MLD injected into the marmosets was ascertained.

*Comparative mortality studies.*—In the experiments in which the mortality ratio of the different yellow fever virus strains in marmosets was studied, large numbers of marmosets were inoculated on the same day with serial dilutions of the virus strain under study and generally two or more strains were used in each experiment. As marmosets are rather delicate animals, and easily succumb to intercurrent infections when first placed in captivity, they were kept in the laboratory a month or more before being used. At first the temperature of the animals was taken twice daily, but this was abandoned when it became evident that marmosets very frequently fail to show a febrile reaction following infection with the virus; there was usually, however, a sharp drop in body temperature shortly before death. The animals were observed daily until their death, or up to thirty days postinoculation. All marmosets were autopsied as soon as possible, but about 90 per cent of them died during the night. In most instances sections of liver, lung, heart, spleen, kidney, and suprarenal gland were taken in 10 per cent formol-saline. The paraffin-embedded sections were stained with H and E.

*Test for the presence of virus.*—To determine the presence of virus in the blood stream, the inoculated marmosets were bled from the heart daily,

and the serum was inoculated intracerebrally in 0.03 ml. amounts into a group of six mice. As marmoset serum is toxic for mice on cerebral inoculation, it had to be diluted; the serum of *C. leucocephala* and *C. jacchus* was diluted 1:3 in saline and that of *L. rosalia* 1:5. For titration purposes serial decimal dilutions of the blood specimen were made in 10 per cent normal monkey serum-saline and each dilution was inoculated intracerebrally in 0.03 ml. amounts into a group of six mice. All mice were observed for thirty or more days and the survivors were reinoculated intracerebrally with approximately  $5 \times 10^2$  MLD of French neurotropic virus. Those which survived this second inoculation were considered to have been immunized by the previous injection, and the titers of the first inocula were calculated as described above under Methods of Infection.

*Tests for the presence of immune bodies.*—Marmosets surviving inoculation were bled around the thirtieth day of the experiment. The pre- and postinoculation serum specimens were tested at the same time in young mice according to the technique of Whitman (5). No marmoset reported here had circulating antibodies prior to the virus inoculation.

### *Virus Strains Employed*

#### *African Strains*

*Asibi strain.*—This strain was isolated in June 1927 by Bauer and Mahaffy from a Negro on the Gold Coast (Stokes, Bauer, and Hudson (1), Sawyer (6)) and since then it has been maintained by passage in monkeys. It is highly virulent for rhesus monkeys, killing about 95 per cent of them. We have worked with the seventeenth, thirty-ninth, fortieth, and forty-third rhesus monkey passages.

*French strain.*—This strain was isolated in December 1927 from a Syrian in Senegal by Mathis, Sellards, and Laigret (7). It had been passed only through monkeys, eleven times at the laboratories of The Rockefeller Foundation in New York, and once in Rio de Janeiro. The number of passages through monkeys prior to arrival in New York is unknown, but is probably not more than four or five. This strain also is highly virulent for rhesus monkeys.

#### *South American Strains*

*J. F. strain.*—This strain was isolated by Soper *et al.* (8) in April 1932 from a Brazilian in the Valle do Chanaan, during the first recognized jungle

yellow fever epidemic. The eleventh monkey passage was used by us. This strain is of low virulence for the rhesus monkey; of the eleven monkeys inoculated, only one died of yellow fever.

*M. A. J. strain.*—This strain was isolated by H. A. Penna in 1935 in Taquaral, State of Goiaz, from a six year old girl suffering from a light case of yellow fever. The strain was established in rhesus monkeys; after the second rhesus passage it was passed three times in mice and since then has been maintained in rhesus monkeys. Of the thirty-six rhesus inoculated, twelve died of yellow fever. The twenty-first rhesus passage was used by us.

*J. Z. strain.*—This strain was isolated by the author in February 1937, in Maracajú, State of Mato Grosso, during an epidemic of jungle yellow fever. Isolation was accomplished in a rhesus monkey, using blood taken twenty hours after onset of illness in a case which was of medium severity and nonfatal. This strain has been passaged only through monkeys and has killed six of twelve rhesus used. The third, fourth, and fifth passages were employed for the marmoset experiments.

*O. C. strain.*—This strain was isolated in January 1940 by Fox and Manso in Santa Leopoldina, State of Espírito Santo, during a jungle epidemic. The case was a very mild one, of only three days duration, and virus was isolated in a rhesus monkey inoculated with blood taken thirty-four hours after onset of the disease. This strain has been passaged solely in rhesus monkeys and has killed seven of the twenty-one animals inoculated. Material representing from the first to fifth monkey passages was used in the present experiments.

*A. C. strain.*—This strain was isolated in a rhesus monkey by L. Whitman in Santa Leopoldina, January 1940, from a light human case. The serum of the original monkey was used by us.

*Martinez strain.*—Isolated in mice from a non-fatal case near Restrepo, Colombia, in 1936 by Kerr and Correa-Henao. After isolation it was passaged twice in rhesus, once through mice, twice through the squirrel monkey (*Saimiri sp.*) and then once in a rhesus monkey. Serum from this last monkey was used by us. Bugher *et al.* (9) state that rhesus monkeys rarely die after subcutaneous inoculation with this strain.

*A. C. Bol. strain.*—This strain was isolated from a severe and fatal case in Santa Cruz de la Sierra, Bolivia, March 1942, by J. Doria Medina. The patient was first seen on the third day of illness, and blood taken fifty hours after the onset of the

disease was inoculated intracerebrally into mice. Mouse brain representing the third passage was sent to Rio de Janeiro and was subsequently inoculated into two rhesus monkeys; both succumbed to yellow fever. Desiccated blood-serum of these monkeys was used in our experiments. Two additional monkeys have been inoculated with this strain and also died of yellow fever.

#### EXPERIMENTAL

##### *Callithrix jacchus* (L.)

This species of marmoset is the most easily obtainable at the local market and therefore was used in the greatest numbers. One hundred and seventy-two animals were used in the different experiments reported here.

##### *Mortality Ratio of C. jacchus Inoculated with Different Yellow Fever Virus Strains*

Groups of marmosets were inoculated with various amounts of virus of several strains and observed for signs of illness. The experiments are given in detail below and the results are summarized in Table I.

*Asibi strain.*—A total of sixteen marmosets, comprising three different experiments, were inoculated with the Asibi strain in its thirty-ninth to forty-third rhesus monkey passage. Three marmosets died after the thirteenth day of the experiment, but none had yellow fever lesions in the liver. Of the two marmosets which received  $1 \times 10^9$  MLD, one became immune and the other did not. All the others survived and became immune.

It was considered possible that the results obtained may have been due to the use of Asibi virus modified by the numerous rhesus passages, and hence recourse was made to the seventeenth passage material, the earliest available. Sixteen marmosets, divided equally between two experiments, were inoculated with virus. Three died with yellow fever lesions in the liver and one died of pneumonia on the ninth day. Eleven of the twelve surviving animals were found to possess antibodies; the serum of the twelfth animal, which had received  $6 \times 10^9$  MLD of virus, was negative for neutralizing antibodies.

The difference in the mortality ratio between the early and late passages of the Asibi virus is of questionable significance, although three marmosets died of yellow fever after the inoculation of the seventeenth rhesus passage virus.

Of the total of thirty-two marmosets inoculated with the Asibi strain, two apparently escaped infection, four died of unknown cause without showing yellow fever lesions in the liver; and only three died of yellow fever. The remaining twenty-three animals became immune.

*French pantropic strain.*—The French strain employed in these experiments was probably not beyond its seventeenth rhesus passage. Twenty-two marmosets were inoculated with graded doses of virus in three different experiments. One animal escaped infection, three died of nonspecific causes, and five succumbed to yellow fever; the

ments were inoculated. Seven of the animals died of yellow fever seven to twelve days after inoculation, three died of unknown cause on the seventeenth and eighteenth days, and five survived and developed humoral antibodies.

*M. A. J. strain.*—In a single experiment, twelve marmosets were inoculated with graded doses of virus representing the twenty-first rhesus passage of this strain. Nine of the marmosets died and three survived and became immune.

*A. C. Bol. strain.*—In two experiments fourteen marmosets were inoculated with graded doses of this strain. Eleven animals died of yellow fever,

TABLE I  
Mortality of *Callithrix jacchus* Inoculated with Different Yellow Fever Virus Strains

NUMBER OF MARMOSETS INOCULATED	VIRUS			RESULTS					
	Strain	Number of passages in rhesus	Range of MLD of virus inoculated	Died of yellow fever		Survived		Non-specific deaths	Yellow fever fatality rate
				Number	A.S.T.*	Number	Number immune		
16	Asibi	39 to 43	$1 \times 10^0$ to $1.1 \times 10^5$	0	∞	13	12	3	0/12 (0 p. c.)
16	Asibi	17	$6 \times 10^1$ to $6 \times 10^5$	3	5.6	12	11	1	3/14 (21 p. c.)
22	French	±17	$2 \times 10^0$ to $1.5 \times 10^5$	5	6.0	14	13	3	5/18 (28 p. c.)
22	J.Z.	3 to 5	$1 \times 10^0$ to $6.6 \times 10^4$	20	7.5	2	2	0	20/22 (91 p. c.)
18	O.C.	1 to 5	$3 \times 10^1$ to $2.7 \times 10^4$	15	8.1	3	2	0	15/17 (88 p. c.)
15	J.F.	11	$2 \times 10^2$ to $1 \times 10^5$	7	9.0	5	5	3	7/12 (58 p. c.)
12	M.A.J.	21	$1.2 \times 10^2$ to $1.2 \times 10^5$	9	7.0	3	3	0	9/12 (75 p. c.)
14	A.C.Bol.	1	$4.8 \times 10^1$ to $4.8 \times 10^4$	11	9.3	1	1	2	11/12 (92 p. c.)
13	Martinez	3	$3.5 \times 10^1$ to $5 \times 10^3$	3	6.6	5	5	5	3/8 (38 p. c.)

\* A.S.T. = Average survival time.

remaining thirteen animals developed neutralizing antibodies.

*J. Z. strain.*—Twenty-two marmosets in four different experiments were inoculated with graded doses of this strain in its third to fifth rhesus monkey passage. Twenty of the animals succumbed to infection and only two, which received  $1 \times 10^2$  and  $1 \times 10^0$  MLD respectively, escaped; when bled later these were found to have developed neutralizing antibodies.

*O. C. strain.*—In three different experiments eighteen marmosets were inoculated with graded doses of this strain in its first, second, and fifth rhesus monkey passage. Fifteen marmosets died of yellow fever. Of the three survivors, two developed antibodies while the third, which presumably received  $2.7 \times 10^1$  MLD, did not.

*J. F. strain.*—Fifteen marmosets in two experi-

two died (ninth and fourteenth days) of nonspecific causes, and one survived and became immune.

*Martinez strain.*—Thirteen marmosets were injected with this strain. Three animals died of yellow fever on the fifth, seventh, and eighth days respectively. Five others died on the sixth to the fourteenth day of the experiment without specific liver lesions, although liver preparations from four of these presented fatty change and two of the four showed a few scattered necrotic cells. The remaining five animals survived and showed circulating antibodies on the thirtieth day of the experiment.

#### *The Circulation of Virus in C. jacchus*

The experiments described above show clearly that marmosets inoculated with most of the jungle strains in contrast to those inoculated with the

Asibi or French strains died as a result of the infection. It was of interest therefore, to compare the amount and duration of circulating virus in marmosets inoculated with strains of African and of South American origin.

Six *C. jacchus* were inoculated in pairs with the following virus strains: Asibi (forty-third passage), J.Z., and O.C. They were bled daily from the first to the seventh day of the experiment to test for virus in the circulation. As can be seen from Table II, virus was present for a considerable length of time in the blood stream of all the animals.

French pantropic, O.C., and J.Z. Each marmoset was bled from the first to the eighth day of the experiment, and the individual daily serum samples were titrated in tenfold dilutions in groups of six mice. As the undiluted serum of this species of marmoset is toxic for mice when inoculated intracerebrally the titrations were started with a  $10^{-1}$  dilution of serum. The results are presented in Figure I.

Both marmosets inoculated with the Asibi strain were found dead on the tenth day; one died of yellow fever and the other apparently died of dysentery.

TABLE II  
*Circulation of Yellow Fever Virus in Callithrix jacchus*

ANIMAL NUMBER	VIRUS INOCULUM		VIRUS IN BLOOD ON POSTINOCULATION DAY							RESULTS			
	Strain	MLD	1	2	3	4	5	6	7	Died		Survived	
										Day of death	Cause of death	Neutralization test	
												Serum specimens	
												Pre-inoculation	Post-inoculation
1	Asibi	$4.5 \times 10^3$	+	+	+	+	+	+	+	20	Unknown; liver negative for yellow fever	—	0
2	Asibi	$4.5 \times 10^2$	—	+	+	+	+	—	—	5	Hemothorax (yellow fever lesions in liver)	—	+
3	J.Z.	$1.5 \times 10^2$	—	+	+	+	+	—	—	5	Hemothorax (yellow fever lesions in liver)	—	0
4	J.Z.	$1.5 \times 10^1$	—	+	+	+	+	+	+	6	Yellow fever	—	0
5	O.C.	$4.5 \times 10^1$	—	+	+	+	+	+	+	7	Yellow fever	—	0
6	O.C.	$4.5 \times 10^0$	—	+	+	+	+	+	+	11	Late yellow fever	—	0

The two marmosets inoculated with the Asibi strain survived the infection. One animal died on the twentieth day of necrosis of the spleen but without yellow fever lesions of the liver, and the other was found to possess neutralizing antibodies in the serum thirty days after inoculation. The daily blood serum samples of these two marmosets never appeared to be icteric.

The remaining four marmosets, inoculated with the J.Z. and O.C. strains, died on the fifth to the eleventh day of the experiment, and were found to have yellow fever lesions in the liver. Generally the blood serum drawn on the day before death or on the day of death was icteric and the blood had a delayed clotting time.

In another experiment four groups of two marmosets each were inoculated with the following virus strains: Asibi (seventeenth rhesus passage),

One of the pair of animals inoculated with the French strain died on the seventh day following a bleeding accident, and only a slight scattered necrosis was seen in histologic preparations of the liver. The other animal survived, with the development of neutralizing antibodies.

All four marmosets inoculated with the O.C. and J.Z. strains died with specific yellow fever lesions in the liver.

Figure 1 shows that virus was present in the blood stream in considerable amounts on two or more consecutive days, and circulated in comparably high amounts after the inoculation of strains of either South American or African origin. However, the length of time that the virus circulated depended on the amount originally inoculated. Use of a large dose of virus resulted in a short period of circulating virus, while inoculation

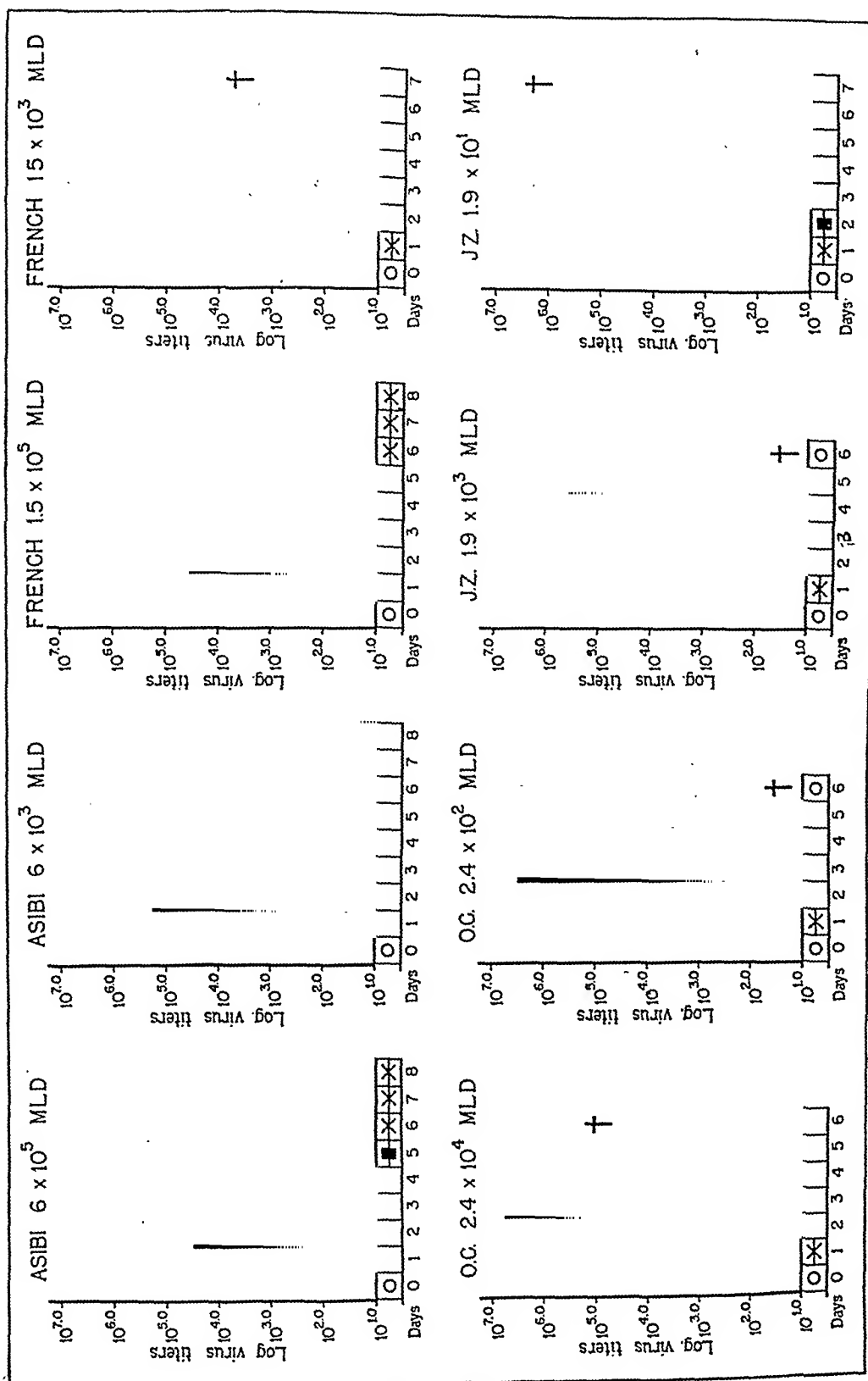


FIG. 1. DAILY TITER OF CIRCULATING VIRUS FOLLOWING SUBCUTANEOUS INJECTIONS OF YELLOW FEVER VIRUS IN *CALLITHRIX JACCHUS*

of a small dose resulted in a much longer period of circulating virus. This is well shown after inoculation of African strains of virus, but is not so apparent with the South American strains, which usually kill the animal.

*Transmission of Virus to C. jacchus by Bite of Mosquitoes*

It was considered possible that parenteral administration of relatively large amounts of virus may have influenced in some degree the outcome of the experiments and hence the effect of a more natural mode of infection was investigated, viz., marmosets were subjected to bites by infected mosquitoes.

*Aedes* (S.) *aegypti* L. were permitted to feed on a rhesus monkey injected with A.C.Bol. virus, and

These experiments show that the method of inoculation had no influence on the outcome of the infection and that marmosets can infect, and be infected by, mosquitoes.

*LEONTOCEBUS ROSALIA* (L).

*Mortality Ratio of L. rosalia Inoculated with Different Strains of Virus*

The pathogenicity for *L. rosalia* of two African and two South American strains of virus was investigated. The experiments, summarized in Table III, are briefly presented below.

*Asibi strain*.—Thirteen marmosets were inoculated with the Asibi strain in its fortieth and forty-third rhesus passage. All the animals survived and when bled thirty days after inocula-

TABLE III  
*Mortality of Leontocebus rosalia Inoculated with Different Yellow Fever Virus Strains*

NUMBER OF MARMOSETS INOCULATED	VIRUS			RESULTS					
	Strain	Number of passages in rhesus	Range of MLD of virus inoculated	Died of yellow fever		Survived		Non- specific deaths	Yellow fever fatality rate
				Num- ber	A.S.T.*	Num- ber	Num- ber im- mune		
13	Asibi	40 to 43	$1 \times 10^4$ to $1.5 \times 10^4$	0	$\infty$	13	13	0	0/13
7	French	$\pm 17$	$1.3 \times 10^4$ to $1.3 \times 10^4$	0	$\infty$	7	5	0	0/5
11	J.Z.	3 + 4	$2 \times 10^9$ to $2.4 \times 10^9$	10	9.4	0	0	1	10/10
4	O.C.	1	$1.5 \times 10^9$ to $1.5 \times 10^9$	4	7.5	0	0	0	4/4

\* A.S.T. = Average survival time.

later were applied individually to a group of four marmosets; one marmoset was bitten by one mosquito, two marmosets by two mosquitoes each, and the last by four mosquitoes. Four days later each marmoset was used to feed one of four different lots of mosquitoes, and afterwards was bled to verify the presence of virus. Virus was present in the blood stream of all the animals, and they died of yellow fever between the fifth and seventh days postinfection.

After a proper incubation period, the four mosquito lots were used to infect six marmosets. Four animals bitten by fourteen, fifteen, seventeen, and eighteen mosquitoes respectively died of yellow fever on the fourth or fifth days of the experiment; the fifth, bitten by seven mosquitoes died of yellow fever on the seventh day and the last, bitten by two mosquitoes, died of yellow fever on the fourth day. Virus was present in the blood stream of all these animals.

tion were found to have developed neutralizing antibodies.

*French pantropic strain*.—Seven marmosets were inoculated with this strain and all survived. Two animals which received the smallest dose ( $1.3 \times 10^1$  MLD) of virus did not develop neutralizing antibodies, while the remaining five did so.

*J.Z. strain*.—Eleven marmosets in two experiments were injected with the J.Z. strain, and all but one died of yellow fever. The exception, which had received  $2.4 \times 10^1$  MLD of virus, died of pneumonia on the twenty-second day.

*O.C. strain*.—Only four marmosets were available, and all died of yellow fever after inoculation with the O.C. strain.

While the number of animals used in these experiments is small, the results are clear-cut: the South American strains were highly lethal for the lion marmoset while the African strains were not.



*Circulation of Yellow Fever Virus in Leontocebus rosalia*

Since in the preceding experiments lion marmosets did not die after inoculation with the Asibi or French pantropic strains, but succumbed to inoculation with the J.Z. or O.C. strains, it was of interest to determine whether this difference was reflected in the amount of virus circulating in the blood. Three marmosets were inoculated with the Asibi strain (forty-third rhesus passage), two with the J.Z. strain, and three with the O.C. strain, and

before, and on the day of death were very icteric, and the blood clotted poorly.

In both animals inoculated with the J.Z. strain, virus circulated in the blood stream from the second to the seventh and last day of the test. One animal died on the seventh day, primarily of a hemopericardium; the serum sample taken on this day was icteric. The other animal was found dead on the morning of the tenth day; blood which had been drawn on the seventh day clotted only partially and the serum was very icteric.

TABLE IV  
*Circulation of Yellow Fever Virus in Leontocebus rosalia*

ANIMAL NUMBER	VIRUS INOCULUM		VIRUS IN BLOOD ON POST-INOCULATION DAY							RESULTS			
	Strain	MLD	1	2	3	4	5	6	7	Day of death	Cause of death	Survived	
												Neutralization test	
												Serum specimens	
												Pre-inoculation	Post-inoculation
1	Asibi	$4.5 \times 10^2$	—	—	—	+	+	+	+	5	.	—	+
2	Asibi	$4.5 \times 10^1$	—	+	+	+	+	+	+	6	Hemopericardium, yellow fever lesions in liver	—	0
3	Asibi	$4.5 \times 10^0$	—	+	+	+	+	+	+	7	Hemopericardium, yellow fever lesions in liver	—	0
4	J.Z.	$1.5 \times 10^2$	—	+	+	+	+	+	+	7	Hemopericardium, hemothorax, yellow fever lesions in liver	—	0
5	J.Z.	$1.5 \times 10^1$	—	+	+	+	+	+	+	10	Yellow fever, yellow fever lesions in liver	—	0
6	O.C.	$4.5 \times 10^1$	—	+	+	+	+	+	+	6	Hemopericardium, hemothorax, yellow fever lesions in liver	—	0
7	O.C.	$4.5 \times 10^0$	—	+	+	+	+	+	—	5		—	+
8	O.C.	$4.5 \times 10^0$	—	+	+	+	+	+	+	5		—	+

bled from the heart daily for seven days to test for the presence of circulating virus. The survivors were bled thirty days later for immunity. Table IV shows the results.

The marmoset which received the largest dose of the Asibi strain showed virus in the blood stream only from the fourth day onwards and survived. The seventh day serum sample was slightly icteric. Two marmosets which received 10 and 100 times less virus, respectively, than the above animal showed circulating virus from the second day until death, which occurred on the sixth or seventh day, and was probably due primarily to a hemopericardium. The serum samples drawn the day

The marmoset receiving the largest dose of the O.C. strain (Table IV) had virus in the blood stream from the second to the sixth day, when it died of a bleeding accident; only partial clotting of the blood occurred and the serum was very icteric. The remaining two marmosets, which received ten times less virus, had virus in the circulation from the second to the sixth, or seventh and last, days of the experiment, survived and became immune.

The results of this experiment indicate that following inoculation of small amounts of these virus strains, the virus appears in the blood within forty-eight hours and persists for days. No

differences were encountered which could be related to the origin of the strain.

#### CALLITHRIX LEUCOCEPHALA (E. GEOFF.)

Only twenty-two marmosets were available for study, and these were tested against the Asibi, J.Z., O.C., and A.C. strains. Twelve marmosets were observed only for signs of infection and the remaining ten were bled daily on the first to the seventh day of the experiment to test for virus in the blood stream.

#### Mortality Ratio of *Callithrix leucocephala* Inoculated with Different Yellow Fever Virus Strains

*Asibi strain*.—Seven marmosets were inoculated with doses ranging from  $1 \times 10^1$  to  $1.5 \times 10^4$  MLD of Asibi virus. Six died of yellow fever on the fifth to the ninth day (average survival time 7.0 days) of the experiment. The seventh animal, which had received  $1 \times 10^3$  MLD, died of unknown cause on the twentieth day.

*J.Z. Strain*.—Only two marmosets were inoculated. These received  $1 \times 10^2$  and  $1 \times 10^3$  MLD of virus respectively and died of yellow fever on the fourth day of the experiment.

*O.C. Strain*.—Three marmosets were inoculated with this strain. Two received  $1.5 \times 10^1$  MLD and the other  $1.5 \times 10^2$  MLD of virus; all died of yellow fever on the fifth and sixth day of the experiment.

#### Circulating Virus in *Callithrix leucocephala*

Ten marmosets were inoculated as follows: two with  $2.4 \times 10^3$  MLD of the Asibi strain, two with  $5 \times 10^1$  and  $5 \times 10^2$  MLD, respectively, of the J.Z. strain, three with the O.C. strain (two with  $3.7 \times 10^3$  MLD and one with  $3.7 \times 10^2$  MLD), and three with the A.C. strain (two with  $4.7 \times 10^2$  MLD and one with  $4.7 \times 10^1$  MLD).

One marmoset, inoculated with  $4.7 \times 10^1$  MLD of A.C. virus, showed a barely detectable amount of virus in the blood on the third day and died on the fourth day with yellow fever lesions in the liver. In the remaining seven marmosets inoculated with the jungle strains, virus appeared in the blood within twenty-four to forty-eight hours and persisted until death, which occurred on the fourth or fifth day (average survival time 4.2 days). Of the two animals inoculated with the Asibi strain, one showed circulating virus from the first to the fourth day, survived, and developed neutralizing

antibodies; the other had virus in the blood from the first to the seventh day (the last tested) and died of yellow fever on the eleventh day.

It thus appears that *L. leucocephala* is highly susceptible to these strains of yellow fever virus.

#### GROSS PATHOLOGY

In all three species of marmoset the liver was generally yellow-red in color, and the lobules were prominent. A yellow-colored liver was encountered only when the animal was exsanguinated. The spleen was usually slightly enlarged. The kidneys were pale and icteric on the cut surfaces. Hemorrhage into the stomach was encountered in a small proportion of cases but usually only a congestion of the stomach mucosa was seen. The other organs were macroscopically normal. Ulceration was seen in a few instances. In general, the clotting time of the blood was greatly reduced.

#### MICROSCOPIC PATHOLOGY

Microscopic examination of liver preparations of marmosets dead of yellow fever revealed at first sight the three degenerative processes associated with yellow fever: necrosis, fatty change, and cloudy swelling.

The necrosis was scattered and necrotic cells were observed in all three zones of the lobules. In liver preparations of *C. jacchus* and *C. leucocephala* the necrosis was evenly distributed throughout the lobule, but in nearly 20 per cent of these cases there were a few lobules in which the necrosis was heavier at the midzone; liver preparations in which the midzonal necrosis of most of the lobules was most marked were exceptional. A few cases were encountered in which the necrosis was more concentrated either at the central or the peripheral zone. In liver preparations of *L. rosalia*, on the other hand, nearly 40 per cent of the cases showed a greater concentration of the necrosis in the midzone.

The degree of necrosis varied from one case to another. In liver preparations of *C. jacchus* and *C. leucocephala* the necrotic cells, also called Councilman's bodies, in general did not have the hyaline aspect and sharp contour encountered in human livers. The nucleus of the liver cells was also necrotic and the remnant could be seen as an acidophilic shadow within some of the necrotic cells. On the other hand liver preparations of *L. rosalia* showed the necrotic cells with a hyaline

aspect and sharp contour as encountered in human yellow fever cases.

Intermixed with the necrotic cells were cells undergoing fatty change. These cells were slightly enlarged and contained large and small droplets of fat. The location in the lobule of the cells undergoing fatty change was variable. The nucleus of the nonnecrotic cells was enlarged and edematous. The chromatin was margined and collected around the nucleolus. Acidophilia of the intranuclear matter was seen.

Inclusion bodies such as those described by Torres (10) occurred with varying frequency, depending on the strain used. The jungle strains generally do not produce intranuclear inclusion bodies in the liver of rhesus monkeys, but it was found that certain of these strains produce inclusion bodies in marmoset (*C. jacchus*) livers. Of the nine liver sections from marmosets inoculated with the M.A.J. strain, six contained intranuclear inclusion bodies; of the twenty-one different liver preparations from marmosets inoculated with the A.C.Bol. strain, inclusion bodies were seen in nine, and of the seven liver preparations with yellow fever lesions from marmosets inoculated with J.F. strain, four contained inclusion bodies. Only in four instances among a total of twenty-five different preparations were inclusion bodies encountered in liver sections of marmosets inoculated with the J.Z. strain. None were found in the liver sections of marmosets which died after infection with the O.C. and Martinez strains. On the other hand the Asibi and the French strains regularly produce intranuclear inclusion bodies in the livers of both rhesus monkeys and marmosets.

Parenchymal cells undergoing cloudy swelling were generally present, and were encountered around the central vein and portal space.

Other changes were a jumbling of the trabeculae, a slight proliferation and hyperplasia of the Kupffer cells, and a slight leucocytic infiltration. Hyperemia of the sinusoids was often encountered. In one case of late death, scattered ocher-stained bodies, such as those described by Villela (11), were seen in the central zone.

#### DISCUSSION

The experiments here presented show that the marmosets *Callithrix leucocephala* (E. Geoff.) and *Callithrix jacchus* (L.) and the lion marmoset *Leontocebus rosalia* (L.) are highly susceptible to yellow fever virus.

The data obtained on *C. jacchus* will be considered first, since the greatest number of marmosets studied belonged to this species. Virus circulated regularly and in considerable amounts in the blood stream of these marmosets, regardless of the virus strain with which they were infected. The mortality, however, depended on the strain employed. The African strains, Asibi and French, were of low virulence for this species of marmoset, producing a mortality of 11 and 28 per cent, respectively. The most virulent jungle strains were the J.Z., O.C., A.C.Bol., and M.A.J. strains, which in *C. jacchus* produced mortalities of 91, 88, 92, and 75 per cent, respectively. The J.F. strain was of medium virulence for *C. jacchus*, as only seven of the twelve animals (58 per cent) died with yellow fever lesions in the liver. The Martinez strain was the most atypical of all these jungle strains. Of the thirteen *C. jacchus* inoculated with this strain, only three died with yellow fever lesions in the liver; five died between the sixth and the fourteenth postinoculation day of unknown cause and five survived and became immune.

It was observed that, with the possible exception of the Martinez strain, there is a marked difference in virulence for *C. jacchus* between the African and South American virus strains tested. While it is possible that the Asibi and the French strains may have been modified by virtue of the seventeen consecutive passages made in rhesus monkeys, the M.A.J. strain, on the other hand, after twenty-one rhesus passages, still proved to be virulent for marmosets. Also, although Stokes, Bauer, and Hudson (1) were unable to isolate either the Asibi or Felice strain in marmosets due to the fact that one survived infection and the other died without specific lesions, the possibility that their marmosets may have been immune to yellow fever cannot be dismissed. These considerations, although not affording direct proof, suggest that the Asibi strain was little if at all modified by the serial rhesus passages and that from the first it was different from most of the South American strains thus far tested.

The lion marmoset *Leontocebus rosalia* was also found to be susceptible to yellow fever virus. As in *C. jacchus*, inoculation of the Asibi or French strains did not produce fatal infections—the virus circulated for considerable periods of time and the animals became immune. Fatal infections, however, were produced by the jungle strains (O.C. and J.Z.).

*C. leucocephala* was highly susceptible to all the

strains tested, and the virus was usually present in the blood stream from the second day until death. The majority of animals died of yellow fever after the inoculation of any of the virus strains tested. However marmosets had a greater average survival time after the inoculation of Asibi virus than that of animals infected with the South American strain.

#### SUMMARY

The marmosets *Callithrix leucocephala* (E. Geoff.) and *Callithrix jacchus* (L.) and the lion marmoset *Leontocebus rosalia* (L.) were found to be susceptible to infection with yellow fever virus.

In *C. jacchus* and *L. rosalia*, death in general did not supervene following inoculation with the Asibi or French pantropic strains; the virus circulated in the blood and neutralizing antibodies subsequently appeared. Most of the South American jungle strains, however, were highly lethal for these species.

In *C. leucocephala*, on the other hand, fatal infections were produced not only by the jungle strains but also by the Asibi strain, the only African strain tested. The average survival time of animals infected with the Asibi strain, however, was greater than that of animals infected with the South American strains.

Specific liver lesions were found in all three species of marmosets following fatal infection with yellow fever virus. These are described and compared with the lesions produced by the virus in the liver of man and rhesus monkeys. Differences between the types of lesions produced in *L. rosalia* on one hand and in *C. jacchus* and *C. leucocephala* are described and discussed.

Marmosets which survived infection with any of the African or South American strains developed a humoral immunity.

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# THE SAIMIRI MONKEY AS AN EXPERIMENTAL HOST FOR THE VIRUS OF YELLOW FEVER<sup>1</sup>

MARSTON BATES

Villavicencio Field Laboratory, Villavicencio, Colombia

The unavailability of rhesus monkeys during the war period has made it necessary to find some other experimental host that can be used for transmission experiments with yellow fever in South America. Because Davis (1930) found that saimiri monkeys were the most susceptible of the South America primates with which he was able to experiment, this animal was among the first that we tried. We have found it to be a very satisfactory host for transmission experiments in so far as it can be infected by very minute doses of virus, usually shows clinical symptoms of infection, and circulates virus with titers comparable to those obtained with rhesus monkeys. It would seem advisable to publish a summary of our observations on virus behavior in this animal as background for adaptation and transmission experiments that will be published later.

## TAXONOMY AND HABITS

Monkeys of the genus *Saimiri* are generally called "squirrel monkeys" in English, and the local name in Colombia is "mico titi." Both terms are frequently applied to other small monkeys—to *Oedipomidas*, for instance, in Panama—so that it would seem advisable to employ the generic name as a common name to avoid possible confusion. The animals with which we have experimented have all come from the region of Villavicencio, in eastern Colombia. This population, according to Dr. George G. Goodwin of the American Museum of Natural History (personal communication) should be known as *Saimiri sciureus caquetensis* Allen. *Saimiri caquetensis* was described by Allen (1916) from Florencia, a town which bears the same relation to the eastern Andes as Villavicencio, but located some 350 kilometers to the south. A discussion of the taxonomy of the monkeys of the *Saimiri sciureus* group has been given by Tate (1939); he makes Kartabo, British Guiana, the

type locality for *sciureus*. The saimiri monkey is a very common animal in the Villavicencio area—probably the commonest local primate—but its distribution in other parts of Colombia is not accurately known. It is interesting that the genus does not reach the Canal Zone in Panama and apparently does not occur anywhere in eastern Panama, though a species known as *Saimiri oerstedii* is found in western Panama (Chiriquí) and in parts of Costa Rica (Goldman, 1921).

Saimiri monkeys live in small bands of fifteen or more individuals, and the bands are commonly accompanied by a few capuchin monkeys (*Cebus*). They are probably omnivorous, but insects are certainly an important part of their natural diet. They are prized as pets in Colombia, and seem to thrive under household conditions. In the laboratory we find that they are not well adapted to life in small cages, and we keep them closely confined only when necessary for experimental work. They live well in a large outdoor cage where there is plenty of room for exercise. The monkeys are given a varied diet of fruits, vegetables, and bread, supplemented by insects as far as practical, and with milk for animals in confinement.

The species is host to a great many internal parasites; we are indebted to Dr. Benjamin Schwartz of the Bureau of Animal Industry for the following identifications. Almost all wild specimens are parasitized by an acanthocephalid worm, *Prosthenorchis elegans*, which is sometimes extremely abundant in the large intestine. Nematodes, apparently of a species of *Filaroides*, are almost always present in the lungs and another nematode, *Dipetalonema gracile*, is sometimes found in the peritoneal cavity. A trematode, *Athesmia foxi*, is frequently found in the liver. These parasites may frequently be a primary or a contributing cause of death among animals in confinement, and they introduce a confusing element into the clinical history of animals infected with virus.

## METHODS OF STUDY

The methods and materials used in this work are those that have become conventional in yellow

<sup>1</sup> The studies and observations on which this paper is based were conducted with the support and under the auspices of the Section of Special Studies maintained by the Colombian Government and the International Health Division of The Rockefeller Foundation.

fever studies. Methods used in studies of native mammals in the Villavicencio area have been described by Bugher *et al.* (1941). The presence of circulating virus has been detected and measured by the intracerebral inoculation of white mice (Theiler, 1930), using the highly susceptible "Swiss strain" (material descended from the stock described by Sawyer and Lloyd, 1931). Titers of circulating virus are calculated from the mouse mortality after inoculation with decimal serial dilutions of serum, according to the method described by Reed and Muench (1938).

Several strains of virus have been tested in saimiri monkeys, though most of the work has been done with various modifications of the "Novoa" strain, isolated from a fatal human infection contracted near Villavicencio. The virulence of a strain for saimiri monkeys undoubtedly depends in part on the immediate anterior history of the strain, and varies considerably. In this connection, however, it should be remembered that our only method of making quantitative estimates of virus in an inoculum or in circulation is by the intracerebral inoculation of white mice, and there is evidence that strains of virus may vary in their virulence for mice. Specificity tests (by incubation of serial dilutions of infected material with known normal and immune rhesus sera) were made several times in the course of the experiments reported here, with results positive for yellow fever in all cases.

Where serum for protection tests or desiccation was needed, the animal was bled from the heart. For checks on circulating virus, animals were bled from the femoral vein or artery without anesthesia—a somewhat difficult procedure because of the small size of the veins. As much as 4 or 5 cc. of blood can be taken from an animal at one time without apparent injury. For routine checks on circulating virus we have, however, taken only 1 or 2 cc. We have not been able to obtain more than 15 cc. from an animal being bled to death from the heart (young individuals). The effect of repeated bleedings on the clinical history of the infected animals is always an unknown factor, and we have consequently bled them only to the extent that seemed necessary for the purposes of particular experiments. This means that our histories of virus circulation are frequently incomplete.

#### CIRCULATION IN VIRUS

We have infected saimiri monkeys by intraperitoneal and subcutaneous inoculation, and by the

bite of the mosquito *Haemagogus capricornii*. The period of virus circulation seems to depend largely on the size of the dose inoculated, but probably also to some extent on the strain of virus and on the characteristics of the individual animal. After inoculation with a massive dose (*i.e.*, on the order of 100,000 m.l.d. for white mice) virus in circulation may reach a maximum titer on the third day after infection and be completely cleared by the fifth day. The appearance of virus in circulation may be greatly delayed after inoculation with a minute dose. This is illustrated by saimiri No. 41 (temperature chart in figure 1) which was inoculated with a trace of virus too small to be detected by the intracerebral inoculation of white mice. Virus measurable by mouse inoculation did not appear in circulation until the fifth day after infection. It seems likely that there was no virus at all in circulation on the fourth day, as 0.5 cc. of serum taken on this day and inoculated in a presumably susceptible saimiri did not result in an infection.

Virus is usually in circulation for three to five days, and the maximum titer, as measured by the inoculation of white mice, frequently exceeds  $1:10^6$ . Such high titers are particularly characteristic of fatal infections; animals that have survived have in no case shown a titer greatly in excess of  $1:10^5$ .

#### TEMPERATURE REACTIONS

Temperature charts of six animals inoculated with various passes of the "Novoa" strain of virus are given in the accompanying charts. The temperature of normal saimiri monkeys is very variable: it may rise to as much as  $40^{\circ}\text{C}$ . with excitement or exercise, and fall to as low as  $36^{\circ}\text{C}$ . when the animals are immobilized, as for feeding mosquitoes. There is apparently a normal daily alternation between a morning low temperature and an evening high. The mean temperatures of five normal monkeys taken over a period of five days while caged in the infected animal room (mean temperature of room about  $27^{\circ}\text{C}$ .) are plotted in figure 1. Each of these five monkeys showed the same type of daily temperature cycle. The mean of the fifty-five readings is  $39.0^{\circ}\text{C}$ . It is hardly possible to speak of "normal" temperature for an animal like saimiri, but  $39^{\circ}\text{C}$ ., at least serves as a convenient standard of reference in comparing charts, and a line has been drawn at this level on all the charts given here.

Temperature charts are given for two monkeys infected by the mosquito *Haemagogus capricornii*: No. 14 by bite and No. 39 by inoculation. Neither

of these monkeys showed a definite fever, but it is possibly significant that in both the daily alternation of high and low temperatures was interrupted during part of the period of virus circulation. The

the mildness of the reaction of monkeys Nos. 14 and 39 were due to the small amount of virus inoculated, we would expect a similar picture in monkey No. 41. This animal failed to show circu-

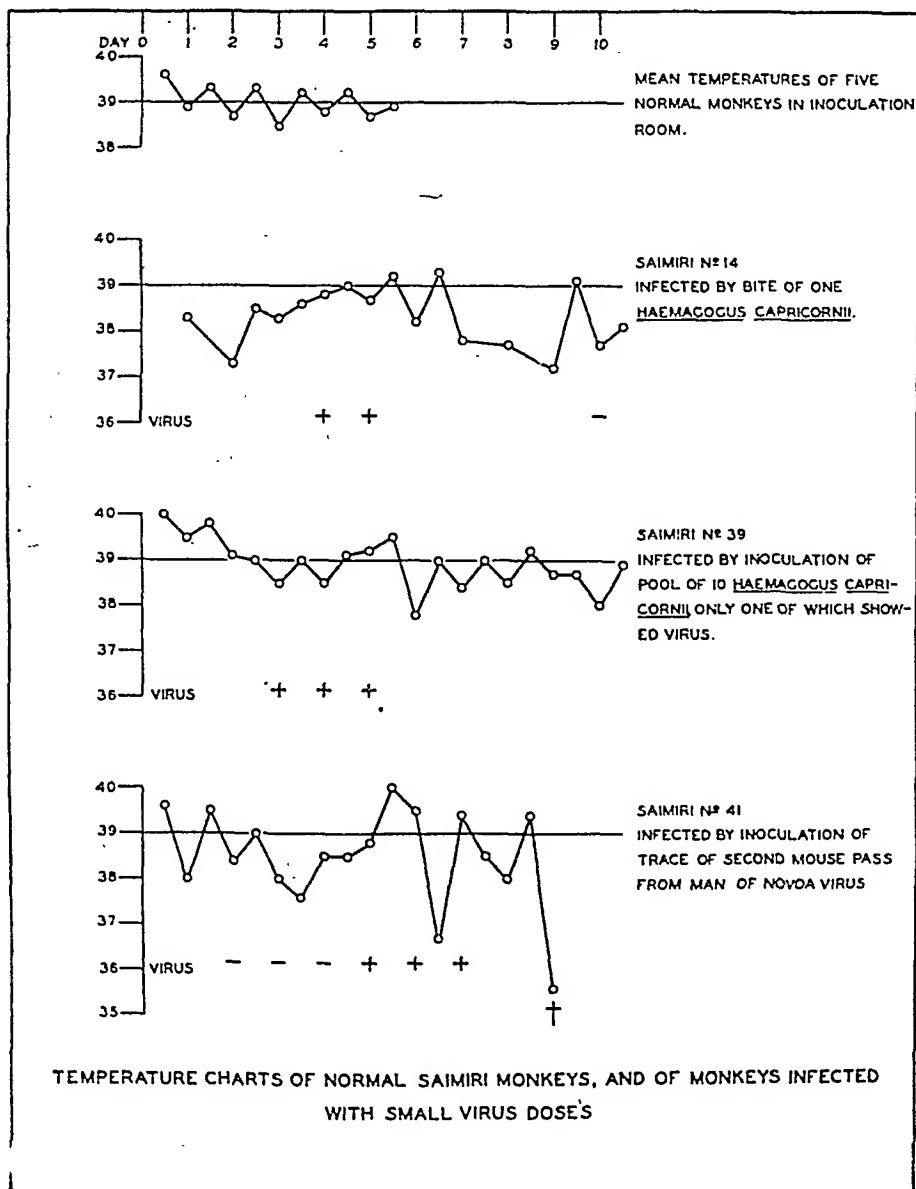


FIG. 1. TEMPERATURE CHARTS OF NORMAL SAIMIRI MONKEYS, AND OF MONKEYS INFECTED WITH SMALL VIRUS DOSES

chart of saimiri No. 41 has been placed with these mosquito infections because the animal was also infected with a trace of virus: in this case too small to be detected by white mice (it received 1.0 cc. of an inoculum that showed no mortality when inoculated in twelve mice in 0.03 cc. doses). If

lating virus until the fifth day and the titer increased steadily until the seventh day, after which it was not bled. It showed definite fever on the fifth day and died on the ninth day; histological examination of the liver showed lesions characteristic of yellow fever.



In figure 2 are given the charts of three monkeys infected in series with a line of virus of the "Novoa" strain that had previously been passed through six brown masked opossums (*Metachirus nudicaudatus*). The monkeys were infected by the intra-

third monkeys it seems probable that both virus and antibodies were circulating on the fourth day, as mouse mortality with pure serum was irregular and with a tenfold dilution regular; by the fifth day virus had disappeared from circulation in both.

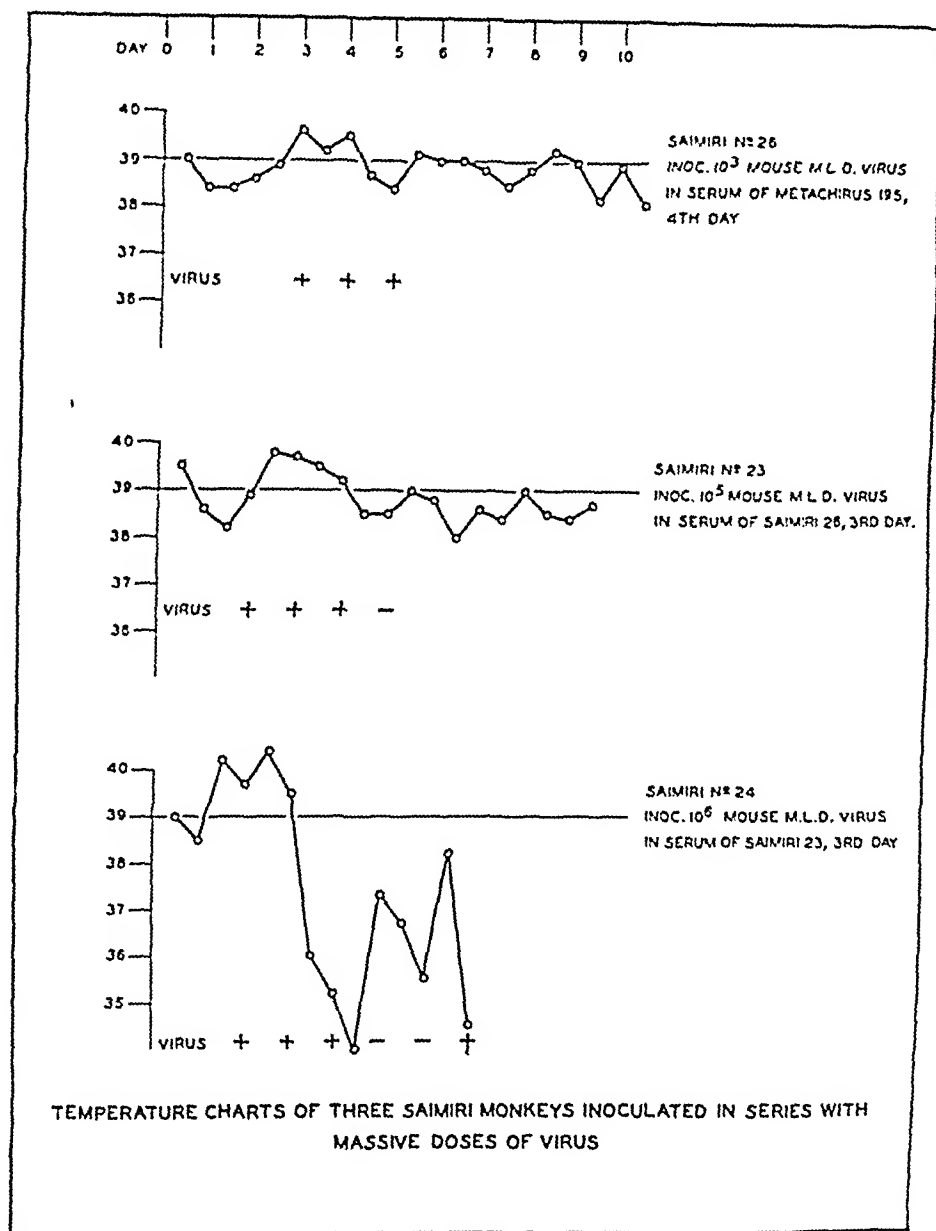


FIG. 2. TEMPERATURE CHARTS OF THREE SAIMIRI MONKEYS INOCULATED IN SERIES WITH MASSIVE DOSES OF VIRUS

peritoneal inoculation of 0.25 cc. of serum taken from the previous animal on the third day after infection: this resulted in the first monkey receiving a thousand m.l.d., the second a hundred thousand, and the third a million. In both the second and

It will be noted that both monkeys Nos. 24 and 41 showed periods of great temperature depression while virus was in circulation. Such extreme fluctuations of temperature are frequent in severe or fatal infections. A period of subnormal tem-

perature may follow directly after the period of fever (as in monkey No. 24) or may be intercalated in the fever period. Death is usually preceded by twenty-four hours or so of subnormal temperature, and the rally shown by monkey No. 24 is exceptional. It should be noted that the subnormal temperature of animal No. 41 on the afternoon of the sixth day corresponded to a period of immobilization for mosquito feeding: such immobilization usually causes a sharp temperature drop, but animals that have not been immobilized may show a similar temperature picture.

Most of our saimiri infections have occurred in the course of transmission experiments in which we did not know in advance whether the inoculum contained virus or not. It has consequently been important to try to determine whether an animal was infected from its clinical history. We have not, however, been able to determine this with any degree of accuracy. Temperature histories similar to those shown by animals Nos. 14 and 39 are frequently obtained from uninfected monkeys, and we have twice secured what seemed sharply characteristic temperature charts (*i.e.*, similar to No. 24) from animals that have not shown virus in circulation. Both of these animals died before they could be tested for immunity, and neither showed diagnostic pathological changes in the liver. We know that saimiri monkeys may be infected with yellow fever when virus is not demonstrable in the inoculum by intracerebral inoculation in white mice and it would seem theoretically possible (though perhaps not probable) that virus of a type nonpathogenic for mice might also circulate.

#### MORTALITY

Fourteen saimiri monkeys infected with known virus have been allowed to follow a natural course of infection except for daily bleedings of 1 or 2 cc. of blood to check on circulating virus. Five of these have died within ten days after infection under circumstances that would seem to indicate virus infection as the primary cause of death. In two of these fatal cases changed blood has been found in the stomach at autopsy. In the experiments of Davis (1930) about half of the animals known to have been infected died, but it is possible that some mild infections escaped notice as the white mouse technique for checking on virus circulation was not then available.

#### PATHOLOGICAL CHANGES IN THE LIVER

Liver tissue of animals killed or dying in the course of infection has been saved, except where

putrefaction had already set in at the time death was discovered. In only one instance have lesions similar to those of man and rhesus monkeys been found; this was in animal No. 41, the temperature chart of which is given in figure 1. None of the other livers showed the intense midzonal necrosis characteristics of yellow fever, though there is usually some pathological change.

#### ANTIBODY PRODUCTION

Serum of all animals has been tested for yellow fever antibodies by the intracerebral protection test as described by Bugher (1940). The tests have been made in the Bogotá laboratory by Dr. Bugher and Dr. Roca, so that results are uniform with those of other animals reported on by them in various papers. It has been impossible to get sufficient serum from these small monkeys to use the standard intraperitoneal test described by Sawyer and Lloyd (1931).

Preinoculation and postinoculation tests on five animals known to have circulated virus are given in table 1. The result is expressed as mouse survival (0/6 means all six mice used were killed, 1/6 that one lived, and so forth), and average survival time of mice. As these tests were made in different "runs," the mouse m.l.d. of virus used in a particular test is given in parentheses. The post-inoculation tests on these five animals show an extremely weak protection, as measured by the intracerebral method. The mouse survival and survival time correspond, in the case of animals Nos. 13 and 14, to a dilution of 1:64 of the immune rhesus serum used as control. It thus appears that only sera with a mouse survival of 0/6 and a survival time of less than 6.0 days can be considered as clear negatives.

We have found no evidence of nonspecific reactions in protection tests with serum of saimiri. This contrasts with certain other groups of mammals in which "false positive" results may be fairly frequent. We have pre-experimental protection tests on the sera of fifty wild monkeys. Of these, forty-one are clearly negative, three clearly positive (5/6 or 6/6 protection), and six doubtful. Most of our monkeys come from an area which has had no history of yellow fever for the past five years. We have not yet been able to accumulate significant numbers of tests from areas with recent history of yellow fever.

We have recovered an abundance of circulating virus in all cases where susceptible animals (*i.e.*, preinoculation protection test apparently negative) have been inoculated with known virus, with

two exceptions. In one of these (saimiri No. 17) the inoculum was rehydrated serum from another saimiri taken on the last day of virus circulation when both antibodies and virus may have been present in blood. Mice inoculated with the pure serum showed irregular mortality while those inoculated with a tenfold dilution showed a more regular mortality. Saimiri No. 17 was bled daily from the third through the seventh day with no virus being recovered; serum taken on the sixteenth day showed a definitely positive protection test result with white mice. This animal may have received a sort of vaccination; or it may have been resistant to infection. In the other anomalous case, an animal with a doubtful preinoculation protection test (0/6 survival, but with an A.S.T. of 7.2 days with 110 m.l.d. of test virus) was

This is about the normal mortality in our laboratory from routine inoculations with nontoxic materials.

To determine whether this toxic effect was a characteristic of the species or of individuals, we made a separate analysis of the results with nine monkeys whose serum was inoculated in thirty or more mice. There was little individual variation. The serum of one young female monkey was consistently less toxic than the average in seven different inoculations over a period of two months (38 per cent mortality in forty-two mice), while the serum of another young female was consistently more toxic than the average (83 per cent mortality in thirty mice), but these were the individual extremes. There seems to be no correlation between toxicity and age or sex.

TABLE 1

*Protection Test Histories of Five Saimiri Monkeys That Showed Circulating Virus*

SAIMIRI NUMBER	VIRUS	VIRUS DOSE	PROTECTION TESTS		NUMBER OF DAYS AFTER INOCULATION WHEN BLED
			Preinoculation	Postinoculation	
13	Infected haemagogus	Inj.	0/6, 5.3 (19)	1/6, 6.8 (250)	21
14	Infected haemagogus	Bite	0/6, 5.3 (88)	1/6, 7.2 (250)	53
18	Asibi strain, i.p.	10 <sup>1</sup>	0/5, 5.6 (19)	3/6, 8.0 (103)	31
23	Metachirus strain, i.p.	10 <sup>1</sup>	0/6, 5.0 (250)	4/6, 9.2 (103)	28
26	Metachirus strain, i.p.	10 <sup>2</sup>	0/6, 4.7 (250)	3/6, 8.5 (103)	14

inoculated with a virus strain known to be highly virulent for rhesus monkeys and white mice. It showed traces of virus in circulation on the third, fourth, and fifth days after infection, and a slight febrile reaction. This is the only case in which we have recovered only traces of virus from a saimiri monkey: usually there is either an abundance of virus in circulation or no virus at all.

#### TOXICITY OF SERUM FOR WHITE MICE

About half of the white mice inoculated intracerebrally with the undiluted serum of saimiri monkeys are killed; the action of the serum seems to be immediate, as most of the mice that are killed never recover from the anesthesia. The toxic action has no relation to the presence of yellow fever virus in the serum. Of 528 white mice inoculated intracerebrally with pure saimiri serum, 308 died within the first 24 hours, giving a mortality of 58.3 per cent. Of 708 mice inoculated with a 1:10 dilution of saimiri serum (in 10 per cent normal human serum in saline), 23 died within the first 24 hours, giving a mortality of 3.2 per cent.

#### DISCUSSION

Saimiri monkeys seem to be the most abundant primates in the region of Villavicencio in eastern Colombia, where there is a history of endemic yellow fever. Under laboratory conditions, the species is highly susceptible to several local strains of virus and to the African Asibi strain (one inoculation, which resulted in a nonfatal infection with a maximum titer of 1:10<sup>5</sup> of virus in circulation). We have been able to carry a local strain of virus from one saimiri to another by the bite of a local forest mosquito (*Haemagogus capricornis*), which would seem to reproduce one transmission mechanism possibly important in the epidemiology of the disease. We have not, as yet, been able to maintain a cycle of such transmissions, perhaps largely because of technical difficulties in handling the mosquitoes.

The laboratory infections of saimiri with yellow fever may readily be grouped into the three categories of mild, moderate, and severe. The temperature charts of monkeys Nos. 14 and 39 illustrate the mild infection, of Nos. 26 and 23 the

moderate infection, and of Nos. 24 and 41 the severe infection. Our series of infections is too small and too heterogeneous to form a valid basis for conclusions as to the nature of this difference in severity, but we have the impression that it depends more on the history of the virus strain than on variation in resistance among individual saimiri. There is considerable evidence for a rapid increase in virulence on serial passage in saimiri. One example is shown by the series of three monkeys of figure 2. In another case, three fatal infections occurred in series after two saimiri-mosquito-saimiri passes, indicating that virulence may build up through mosquito passes as well as through direct transfer of infected serum. There was on the other hand, no apparent increase in virulence for saimiri after six passes by transfer of serum through the marsupial *Metachirus*, as the strain used in this experiment caused nonfatal infections in saimiri both before and after the *Metachirus* passes.

#### SUMMARY

The Villavicencio population of *Saimiri sciureus caquetensis* is highly susceptible to the virus of yellow fever: nineteen animals with negative pre-inoculation protection tests have been inoculated with known virus, and circulating virus has been recovered in all except one case (an animal that may have received a mixture of virus and antibodies). The maximum titer of virus in circulation is usually at least 1:100,000 and in acute infections exceeds 1:1,000,000.

The mortality rate in a series of fourteen infections with various strains of virus was about 33 per cent. There are usually some pathological changes in the liver in fatal infections, but lesions characteristic of yellow fever as found in man and rhesus monkeys have only been seen in one case. There is usually a clear febrile reaction to the infection.

The species may be infected by doses of virus too small to be detected by the standard method of intracerebral inoculation in susceptible white mice.

Antibody production, as measured by the intracerebral protection test, may be very weak, corresponding to a dilution of 1:64 of immune rhesus serum. There is, however, no evidence of "false positive" reactions.

The advantages of saimiri for the laboratory study of yellow fever virus are: availability (over much of South America), susceptibility, and the relative ease with which the animals can be maintained in captivity.

Disadvantages are: small size, high degree of helminth parasitism, toxicity of pure serum for white mice, and the uncertainty of working with animals that may have been exposed to infection in the wild state before capture.

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# EXPERIMENTS WITH THE VIRUS OF YELLOW FEVER IN MARSUPIALS, WITH SPECIAL REFERENCE TO BROWN AND GREY MASKED OPOSSUMS<sup>1</sup>

MARSTON BATES

Villavicencio Field Laboratory, Villavicencio, Colombia

The experiments reported by Dr. John C. Bugher and his colleagues (1941) indicated that marsupials as a group might be of considerable interest in relation to the epidemiology of jungle yellow fever. Their results were, however, anomalous in that only a small proportion of the animals were found to develop antibodies after inoculation, while an even smaller proportion showed circulating virus. There was some indication of specific differences in susceptibility, virus being recovered from a higher proportion of certain species than of others. Further investigation of the nature of these differences in susceptibility seemed warranted, and work was undertaken to explore the possibility of virus adaptation to marsupial hosts through serial passage of virus. This work was started at the suggestion of Dr. Bugher, and we are indebted to him for much helpful advice and criticism; we have also had access to the original data and notes of his own experiments, and we have at times drawn upon these in the preparation of the present paper. Dr. Henry W. Kumm, Dr. Manuel Roca-Garcia, and Dr. Jorge Boshell-Manrique have all taken a keen interest in the work, making many valuable suggestions. All protection tests were carried out in the Bogotá laboratory under the direct supervision of Dr. Bugher and Dr. Roca.

## TAXONOMY

A series of skins and skulls of Villavicencio mammals has been saved for subsequent taxonomic study, in the hope of defining the morphological characteristics of the local populations as closely as possible. In the meanwhile, Dr. George Goodwin of the American Museum of Natural History (personal communication) considers that the following population names are probably applicable to the marsupials considered in this paper:

<sup>1</sup> The studies and observations on which this paper is based were conducted with the support and under the auspices of the Section of Special Studies maintained by the Ministry of Labor, Hygiene, and Social Welfare of the Republic of Colombia and the International Health Division of The Rockefeller Foundation.

*Didelphis marsupialis marsupialis* Linnaeus.

This is the "common opossum" of lowland tropical America, the representative of the Virginia opossum of the United States. It is known locally as "*chucha comun*."

*Metachirops opossum griseescens* Allen.

*Metachirus nudicaudatus columbianus* Allen.

There seems to be no consistent usage of English names for these opossums. Both *Metachirops* and *Metachirus* are sometimes called "four-eyed opossums" and sometimes "masked opossums," referring to the light spots above the eyes. The latter term seems somewhat more euphonious. *Metachirops*, with this nomenclature, would be called the "grey masked opossum" and *Metachirus* the "brown masked opossum." The local name for both is "*chucha real*," and local hunters seem not to distinguish between the two.

The animals are closely similar, as can be seen from the photographs (figs. 1 and 2). The two species were included in the same genus (*Metachirus*) until 1916, when Matschie (for taxonomic references see Tate, 1939) separated the grey species as *Metachirops*. He based this generic separation on "the presence or absence of pouch, on the degree of pilation of the base of the tail, and on the presence or absence of postorbital processes" (Tate, 1939).

*Caluromys laniger cicur* Bangs.

The various races of *laniger* are uniformly called "woolly opossums" in English. The local name in Villavicencio is "*chucha mantequera*." A photograph of the animal has been included (fig. 3) to show how much it differs in appearance from the two masked opossums.

Tate (1939) discovered technical grounds for using the generic name *Philander* for the grey masked opossums; but as the name *Philander* had previously been consistently used for the woolly opossums he considered that it was inadvisable to shift the names "pending some opinion from the International Commission." In so far as Tate's



FIG. 1. Grey Masked Opossum (*Metachiroptis opossum*)



FIG. 2. Brown Masked Opossum (*Metachirus nudicaudatus*)



FIG. 3. Woolly Opossum (*Caluromys laniger*)

paper on the mammals of the Guiana region forms a convenient guide to the similar Villavicencio fauna, it seems best to follow his nomenclature. The history of these generic names in recent papers on northern South America and Panama is summarized in table 1. The references in Goldman and Enders, incidentally, contain much information of interest on the behavior and habits of the animals.

The question of the degree of relationship between the grey masked opossum (*Metachirops*) and the brown masked opossum (*Metachirus*) is of considerable interest in the present connection because the two animals—so similar in appearance and structure—differ greatly in their susceptibility to the virus of yellow fever. The case is similar to that described by Findlay and Mahaffy (1936) for African and English hedgehogs. It seems possible that, outside of the primates, suscepti-

has usually been removed by cardiac puncture. For daily checks on circulating virus we have found it possible to bleed the animals from the femoral vein or artery, though the operation is uncertain and tedious. For protection tests with the masked opossums we have removed 3 cc. of blood; where serum for desiccation was needed, we have removed 7 cc. Death is, however, very frequent when this amount is taken. It is rarely possible to get more than 10 cc. when the animal is bled to death. Much larger amounts of blood can, of course, be taken from the common opossum (*Didelphis*).

#### *Preliminary Attempts at Serial Passage of Virus*

We first attempted serial passage of virus in marsupials by the blind transference of serum on the 5th day of infection through series of 3 animals. Such a series was made with the grey masked

TABLE 1  
*Recent history of generic terms for the masked and woolly opossums*

TATE (1939) AND PRESENT PAPER	ASSIGNED TO FOLLOWING GENERA BY:			
	Allen (1916) Goldman (1920)	Enders (1935)	Bugher (1940)	Bugher <i>et al.</i> (1941)
<i>Metachirus nudicaudatus</i> .....	Metachirus	Metachirus	Metachirus	Metachirus
<i>Metachirops opossum</i> .....	Metachirus	Metachirops	Metachirops	Philander
<i>Caluromys laniger</i> .....	Philander		Philander	Caluromys

bility to yellow fever is a highly specific characteristic of animal populations, not in any way correlated with the phylogenetic relationships of the populations.

#### MATERIALS AND METHODS

The materials and methods used in the present study were in general the same as those described by Bugher *et al.* (1941) and Bates (1944). All of the animals were caught by trapping, mostly in the region of Restrepo (see map, p. 312, Bugher *et al.* 1941), where there has been no known human yellow fever since 1938. The animals have been inoculated either intramuscularly, subcutaneously, or intraperitoneally. We have noted no difference in infection among these inoculation routes. Virus dosage, where given, is always estimated in terms of mouse m.l.d., using the 50 per cent end point method described by Reed and Muench (1938).

Animals have been bled for a preinoculation protection test immediately before infection. Blood

opossum (*Metachirops*), the 1st animal being infected by the subcutaneous inoculation of 10 m.l.d. of "Martinez" virus (the strain used in the experiments described by Bugher *et al.*, 1941); transfers were made by the subcutaneous inoculation of 0.03 cc. of serum to successive animals. No virus was recovered in any case. Two series were made with the common opossum. In the 1st series young animals raised in the laboratory were used; the 1st animal (*Didelphis* No. 115) was given a massive intraperitoneal inoculation of "Martinez" virus (100,000 m.l.d.). This animal showed circulating virus with a titer in excess of 1:100 on the 5th day. It died in bleeding, and 0.5 cc. of a liver suspension was inoculated intraperitoneally into a 2nd *Didelphis*; this animal showed no circulating virus. Blind passes on the 5th day were continued through 2 more animals but virus was not certainly recovered again. A 2nd series of blind passes, starting with "Martinez" virus, was made through 3 animals without recovering virus.



An attempt was made with the brown masked opossum (*Melachirus*) at the same time as these 3 attempts with *Melachirops* and *Didelphis*. The 1st animal was inoculated with 0.5 cc. of "Martinez" virus (the equivalent of about two million mouse m.l.d.), and the serum was passed blindly to 2 new animals in series. By the time the 3rd animal was bled it had become apparent that the mouse controls of the 1st animal were going to be negative, so passes were discontinued. We found, however, that although the serum of the 1st animal taken on the 4th day showed no virus on mouse inoculation, it nevertheless had served to infect the 2nd animal (0.5 cc. serum inoculated intraperitoneally) which showed a titer of virus of 1:100 on the 5th day after infection, when its serum was passed to the 3rd animal. The 3rd animal showed a trace of circulating virus. We attempted to recover this strain by inoculating brain suspension of the mouse controls of the 3rd animal into a 4th, but virus was not recovered again.

These exploratory experiments indicated that it might be possible to pass virus in series through the brown masked opossum, but that such passage through the grey species or the common opossum would be very difficult because of the irregularity of infection. They also seemed to indicate that the virus might show an adaptation to the brown masked opossum that was lost through a mouse brain passage. The brown masked opossum, from these and from other infection experiments, seemed the logical animal for test of this adaptation theory. We waited until we had accumulated a fair number of animals with which to experiment, and then started on a new attempt at serial passage of virus.

#### SERIAL PASSAGE OF VIRUS IN THE BROWN MASKED OPOSSUM

We selected the "Novoa" strain of virus for the 2nd serial passage experiment with this species because all manipulations since the isolation of the strain from man had been made in this laboratory, so that we had a complete record of its behavior. We started with rehydrated serum from a Saimiri monkey which had been infected by material of the 5th mouse pass of this strain, as shown in the accompanying diagram. Mosquitoes infected on this monkey had given us our first laboratory transmission of yellow fever through *Haemagogus capricornii*, so that it seemed likely that the strain

was fairly "normal" despite the 5 mouse brain passes.

The plan of the experiment was as follows: infection was to be by the inoculation of 0.06 cc. (2 mouse doses) of serum intramuscularly (using a 25 gauge needle and inoculating in the thigh, on a theory that this would approximate "natural" inoculation); the animals would be bled uniformly on the 4th day for passage, as exploratory experiments indicated that virus was most often circulating on this day. We started by inoculating 3 animals, passing the serum directly on the 4th day to 3 new animals, and so for 3 passes; serum of the 3 animals of the 3rd pass was desiccated and held until mouse results were available. Serum of No. 180 (see diagram) was also passed blindly to No. 181, and so for 2 more passes; as will be seen in the diagram, the virus was lost in animal No. 182. Serum of No. 180 was then rehydrated and inoculated into 4 animals. Serum of these, taken on the 4th day, was desiccated and held until mouse results were available. Serum of the animal showing the highest titer was then rehydrated and inoculated into 4 more animals. This process was repeated from the 10th through the 13th laboratory passes of the virus, as shown in the diagram. At this stage we again tried 2 series of blind passes (from animals 201 and 202), but the virus was lost (or at least became non-infective for mice) in the 3rd pair of animals (16th laboratory pass of the diagram). We thus had 10 consecutive passes in the brown masked opossum, which seemed sufficient for studying possible adaptation of this strain to that species of mammal.

Serum of No. 195 was inoculated into a Saimiri monkey, and blind passes were continued through 2 more Saimiris. Virulence increased rapidly during these 3 passes, and the 3rd animal died. Its serum was titrated with normal and immune rhesus serum in a specificity test, which was clearly positive for yellow fever. Serum of No. 215, at the end of the series of opossum passes, was inoculated into another Saimiri monkey. Although this *Melachirus* serum was non-infective for mice, the Saimiri was infected and showed circulating virus on mouse inoculation; a specificity test was made with this mouse brain material, which was positive for yellow fever. The immunological properties of the virus had thus not changed at the end of the experiment.

This serial passage experiment seems sufficiently interesting and important to warrant a detailed

treatment of the experimental data. We have tried to give a complete summary of the data in the diagram and in table 2. The protection test results of table 2 will be discussed later. Data

tions. We have calculated titers from these dilutions according to the method of Reed and Muench (1938), and these titers form the basis of the estimate of virus dose used as inoculum. Since

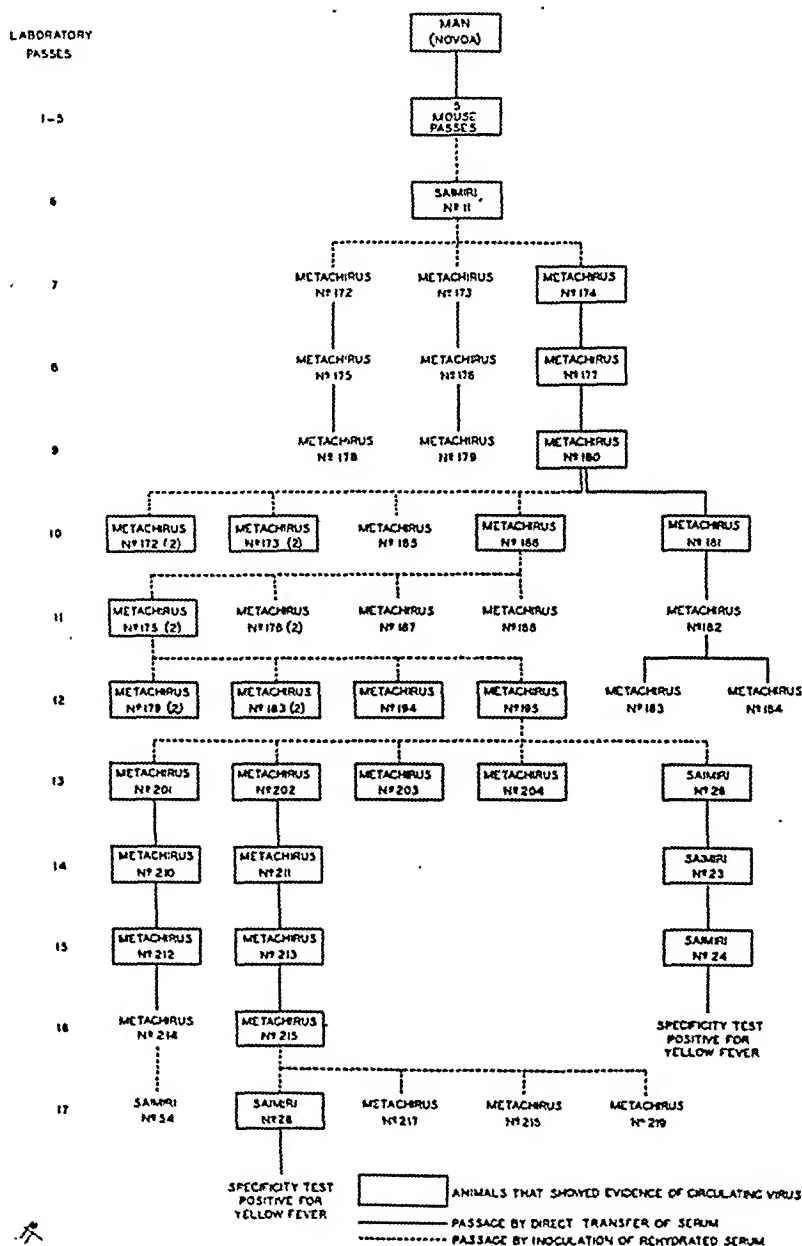


Diagram of Virus Passage Experiment with Metachirus

are given for bleedings on the 3rd, 4th, and 5th day after infection; a few animals were also bled on the 2nd and 6th days, but no animal showed virus on these days alone. Mouse inoculations were made of pure serum and of 3 tenfold dilu-

only 6 mice were inoculated with each dilution of serum, and since mouse infection with marsupial serum seemed at times somewhat irregular, these titers must be regarded as of dubious significance. For this reason we have indicated titer in the table

as "+" when mice were killed only by the undiluted serum, "++" when mice were killed by *Metachirus* 215, which showed no virus on white mouse inoculation, was nevertheless infective for a

TABLE 2  
*Histories of Metachirus used in serial passage experiment*

METACHIRUS NUMBER	PREINOCULATION PROTECTION TEST			DOSAGE OF VIRUS EXPRESSED AS M.L.D. MOUSE	PRESENCE OR ABSENCE OF CIRCULATING VIRUS			DAY OF DEATH	POSTINOCULATION PROTECTION TEST		
	Survival ratio	Average survival time	M.L.D. mouse		3rd day	4th day	5th day		Survival ratio	Average survival time	M.L.D. mouse
172	0/6	5.3	19	1	0	0	—		0/6	5.8	19
172 (2)	0/6	5.8	19	250	—	+	0		0/6	6.7	110
173	1/6	6.0	19	1	—	0	—		0/6	5.7	19
173 (2)	0/6	5.7	19	250	—	0	+	6th	3/6	8.2	19
174	0/6	5.3	19	1	—	++	—		1/6	5.7	19
175	0/6	5.0	19	0	—	0	—				
175 (2)	1/6	6.0	110	10	++	+++	—	5th	5/6	9.3	19
176	1/6	6.5	19	0	—	0	—		6/6	10.0	110
176 (2)	4/5	9.8	110	10	0	0	—		1/6	6.2	19
177	0/5	5.6	19	90	—	++	—				
178	1/6	6.3	19	0	—	0	—	30th	0/5	5.0	19
179	0/5	5.4	19	0	—	0	—				
179 (2)	0/5	5.0	19	4	—	++	—	4th	5/6	9.5	250
180	0/6	5.0	19	20	—	++	—		4/5	9.0	110
181	1/5	6.0	19	82	—	+	—				
182	0/5	5.4	19	tr.	—	0	—	5th	4/4	10.0	110
183	1/6	6.8	19	0	—	0	—		4/6	9.5	103
183 (2)	4/4	10.0	110	4	—	++	—				
184	2/6	7.2	19	0	—	0	—	25th			
185	0/6	5.3	19	200	—	0	0	17th			
186	0/6	6.0	19	250	—	++	0	21st	0/6	5.3	110
187	0/6	5.7	110	10	0	0	—				
188	0/6	5.0	110	10	0	0	—	5th			
194	0/6	5.2	110	4	++	++	—	4th			
195	0/6	5.2	110	4	++	+++	—	4th	5/6	9.7	103
201	0/6	5.5	103	100	++	++	++				
202	0/6	5.5	103	100	++	+++	—	4th	2/5	8.0	103
203	0/6	4.8	103	100	++	+++	—		1/4	7.5	103
204	0/6	5.7	103	100	—	0	+		0/6	5.7	103
210	0/6	5.5	103	10	—	+++	—		2/6	8.0	103
211	0/6	5.2	103	10	—	++	—		2/6	7.3	20
212	0/5	5.8	103	450	++	+	—				
213	0/6	5.3	103	6	++	+++	—				
214	0/6	4.7	103	1	0	0	—	4th			
215	0/6	5.7	103	2000+	+	0	—	4th			
217				tr.	0	0	0				
218				tr.	0	0	0	27th			
219				tr.	0	0	0				

Notes: Tr. = less than 1 mouse m.l.d. of virus; — = not bled; 0 = bled, but no demonstrable virus; + = control mice killed by pure serum only; ++ = mice killed by serum diluted 1:10 and 1:100; +++ = mice killed by serum diluted 1:1000.

dilutions of 1:10 and 1:100, and "+++" when some mice were killed by serum diluted 1:1000. It is interesting that the 4th day serum of

Saimiri monkey. Forty-eight white mice have been inoculated with this serum at one time or another without the recovery of virus in any case.

The 3 opossums (217, 218, and 219) inoculated with this serum did not show circulating virus; No. 217, however, circulated virus after a later inoculation with rehydrated serum of No. 203, so the first inoculation was surely non-infective. The Saimiri monkey showed a very delayed infection (virus first in circulation on the 5th day), indicating the inoculum had contained only a minute trace of virus. We at first thought that the virus of No. 215 might have shown a qualitative change due to the repeated opossum passage, rendering it non-infective for white mice; but

did successive animals show regularly increasing series of titers of circulating virus.

#### *Other Infection Experiments with Brown Masked Opossums*

A few miscellaneous *Metachirus* infections are summarized in table 3. Circulating virus was recovered from 9 of 13 animals in these experiments. Of 29 inoculations in the serial passage experiment in which the inoculum contained demonstrable virus, the virus was recovered in 21 cases. Combining these 2 series we get a total

TABLE 3  
*Miscellaneous infections of Metachirus*

METACHIRUS NUMBER	PREINOCULATION PROTECTION TEST			VIRUS STRAIN	DOSAGE OF VIRUS EXPRESSED AS M.L.D. MOUSE	PRESENCE OR ABSENCE OF CIRCULATING VIRUS				POSTINOCULATION PROTECTION TEST		
	Survival ratio	Average survival time	M.L.D. mouse			3rd day	4th day	5th day	6th day	Survival ratio	Average survival time	M.L.D. mouse
111	0/5	6.0	135	Martinez	10 <sup>6</sup>	—	0	+	—			
124				Met. 111	tr.	—	—	++	0	6/6	10.0	25
129				Met. 124	1000	—	—	+	—	6/6	10.0	25
131	5/6	9.3	25	Martinez	±10 <sup>6</sup>	—	—	+	—			
133	0/6	5.0	63	Martinez	50,000	—	—	+	—			
171	0/6	5.0	19	Martinez	800	—	—	0	—			
200	0/6	5.8	110	Asibi	5,000	—	0	—	—	1/6	7.2	103
220				Volcanes	2,000	++	+++	+	—			
221				Volcanes	2,000	++	0	0	—			
222				Met. 203	6	—	0	—	—			
223				Met. 203	6	—	0	0	++			
217 (2)				Met. 203	6	++	+++	++	0			
219 (2)				Met. 203	6	0	0	0	—			

Notes: Tr. = less than 1 mouse m.l.d. of virus; — = not bled; 0 = bled, but no demonstrable virus; + = control mice killed by pure serum only; ++ = mice killed by serum diluted 1:10 and 1:100; +++ = mice killed by serum diluted 1:1000.

there is no evidence for this. Saimiri monkeys are apparently susceptible to virus doses too small to be detectable on mouse inoculation (Bates, 1944), and the present case seems merely to be another example of the sensitivity of these monkeys.

It is clear that virus was carried through 10 serial passes in the brown masked opossum, and that it was infective for Saimiri monkeys after 6 passes and after 10 passes. It is not, however, clear that there was any measurable adaptation to the marsupial host in the course of these 10 passes. Infection seemed much more regular after the 5th pass, but the strain was lost in opossums at the 10th pass, and nowhere in the experiment

rate of virus recovery of 72 per cent. It seems likely that in many cases circulating virus was overlooked through failure to bleed on the proper day. In other cases, it seems certain that the animal did not receive an infective dose, as virus was circulated on reinoculation. We have not noted any clinical symptoms of virus infection in this species. Death could usually be ascribed either to helminth parasitism or to injury incident to bleeding.

#### *Infection Experiments with Grey Masked Opossums*

Infection experiments with 28 grey masked opossums are summarized in table 4. The data have been given in detail because of the irregular times at which animals have been bled for circu-

lating virus. It is, of course, perfectly possible that an animal bled on the 4th day only showed circulating virus at some other time; bleedings have usually been made on this day because virus was recovered most frequently from the brown masked opossum after that interval. It will be noted that virus was certainly recovered from only

In several cases the 2 masked opossums were inoculated at the same time with the same virus dose. These cases are tabulated in table 5.

Bugher *et al.* (1941) report the recovery of circulating virus from 3 of 15 specimens of the grey masked opossum; we have recovered virus from 1 of 28. It is obvious from this that the grey species

TABLE 4  
*Histories of Metachirops inoculated with yellow fever virus*

META- CHIOPS NUMBER	PREINOCULATION PROTECTION TEST			VIRUS STRAIN	DOSAGE OF VIRUS EXPRESSED AS M.L.D. MOUSE	PRESENCE OR ABSENCE OF CIRCULATING VIRUS				POSTINOCULATION PROTEC- TION TEST		
	Survival ratio	Average survival time	M.L.D. mouse			3rd day	4th day	5th day	9th day	Survival ratio	Average survival time	M.L.D. mouse
100				Novoa	5,000	—	—	—	0	0/6	6.0	135
101	5/6	9.3	135	Novoa	5,000	—	—	—	0	0/4	6.5	135
103	1/6	7.7	135	Martinez	10	—	—	0	—			
104				Martinez	10 <sup>6</sup>	—	—	0	0	2/4	8.0	25
135	3/6	8.0	25	Martinez	50,000	—	—	++	—			
168	0/6	6.0	19	Martinez	800	—	—	0	—	2/6	7.3	110
169	3/5	9.0	19	Novoa	60,000	—	—	0	—	1/6	6.3	110
189	0/5	7.4	110	Novoa	2,000	—	0	—	—	0/6	5.7	103
190	1/5	6.8	110	Novoa	2,000	—	0	—	—			
191	1/5	7.2	110	Novoa	2,000	—	0	—	—	2/6	7.0	103
192	3/5	8.0	110	Novoa	2,000	—	0	—	—	0/6	6.2	103
193	1/6	6.8	110	Novoa	2,000	—	0	—	—	0/6	5.7	103
196	0/6	5.5	110	Asibi	5,000	—	0	—	—	0/6	6.3	103
197	0/6	5.7	110	Asibi	5,000	—	0	—	—	1/6	6.7	103
198	0/6	6.0	110	Asibi	5,000	—	0	—	—			
199	0/6	5.2	110	Asibi	5,000	—	0	—	—	0/6	4.8	103
205				Met. 195	100	0	0	0	—			
206	0/6	6.0	103	Met. 195	100	0	0	0	—	0/6	5.0	103
207	0/6	5.3	103	Met. 195	100	—	0	0	—	0/6	5.3	103
208	0/6	7.3	103	Met. 195	100	—	?	0	—	0/6	7.2	103
209	0/6	6.7	103	Met. 195	100	—	0	0	—	0/6	6.0	103
210				Volcanes	2,000	0	0	—	—			
211				Volcanes	2,000	—	0	—	—			
212				Volcanes	2,000	—	0	—	—			
213				Volcanes	2,000	—	0	—	—			
214				Met. 203	6	0	—	—	—			
215				Met. 203	6	0	0	0	0			
216				Met. 203	6	—	0	0	0			

1 animal (No. 135); this was following intraperitoneal inoculation with a large dose of "Martinez" virus after its 2nd pass through Saimiri monkeys. Mouse mortality from the inoculation of serum of this animal was very irregular: death occurred in 2 of 6 mice inoculated with pure serum, 5 of 6 inoculated with 1:10 dilution, and 2 of 6 inoculated with 1:100 dilution; the mouse mortality was regular on passage of brain material from the paralyzed mice.

must be classed as refractory to infection with the virus of yellow fever of the strains used and under the conditions of these experiments.

#### *Antibody Response as Measured by the Intracerebral Protection Test*

Protection test results have been given in the various animal histories tabulated in preceding pages of this paper. The tests were carried out with the intracerebral method of Theiler (1933)

as described by Bugher (1940). Essentially, serum to be tested is incubated for 2 hours at a moderate temperature (27° to 30°C.) with an equal amount of a known dilution of neurotropic virus; the mixture is then inoculated intracerebrally into susceptible white mice in 0.03 cc. amounts, together with appropriate controls. The advantage of this method over the standard intraperitoneal method described by Sawyer and Lloyd (1931) is that tests can be made with relatively small amounts of serum. Usually 6 or 12 mice are inoculated with a given serum-virus mixture; if the serum contains no virucidal properties, all the animals die on the 4th or 5th day after inoculation; if virucidal properties are present, the death of the mice may be

TABLE 5

*Parallel infections of Metachirus and Metachirops*

VIRUS STRAIN AND DOSAGE (MOUSE M.L.D.)	METACHIRUS		METACHIROPS	
	Num- ber inocu- lated	No. circu- lating virus	Num- ber inocu- lated	No. circu- lating virus
Asibi, 5,000 m.l.d., in- traperitoneally.....	1	0	4	0
Volcanes 2,000 m.l.d., intraperitoneally.....	2	2	4	0
Metachirus 195 100 m.l.d., intramuscularly	4	4	5	0
Metachirus 203 6 m.l.d., intramuscularly.....	2	1	3	0
Total.....	9	7	16	0

delayed or none may be killed. Tests are run for 10 days, and the result is expressed as a fraction indicating the number of mice living at the end of the 10-day period, and by a number representing the mean number of days that the mice lived. Thus a result of "0/6, 4.5" indicates that all mice were killed, and that the average survival time was 4.5 days. "6/6, 10.0" would indicate that all mice lived; since the test is discontinued at 10 days, the average survival time is 10.0. In so far as it is apparently impossible to achieve complete standardization of virus dose, the dosage actually used in a particular test has been added in the case of all tests listed in this paper. Tests of sera reported on here have been run with virus doses varying from 19 m.l.d. to 250 m.l.d., and such variation naturally results in differences in mouse survival, though the differences due to this factor seem to be much less than one might expect.

The "interpretation" of a particular test run must always be made with reference to titrations of a standard pool of known immune serum run in a control series. In theory a completely negative result would be "0/6, 4.5" and a completely positive result "6/6, 10.0."

The intracerebral protection test seems to be a reliable guide to infection in man (Theiler, 1933), and we have found it to be a reliable guide in work with Saimiri monkeys (Bates, 1944), only 12 per cent of the results with sera of 50 wild animals being "doubtful." The results with marsupial sera are, however, very puzzling. The contrast between the brown masked opossum (*Metachirus*) and the grey masked opossum (*Metachirops*) is particularly striking in this regard. The majority of the brown masked opossums with which we have experimented show perfectly "normal" behavior: that is, an animal with a "negative" pre-inoculation protection test will show circulating virus when inoculated with virus, and a protection test with its postinoculation serum will show definite protective power—a "positive" result. With the grey masked opossum, however, we can detect no relation between the protection test result and exposure to the virus of yellow fever, and even with the brown species there are a striking number of anomalous results.

The average survival time of the mice used in the test is considered by Bugher (1940) to be a reliable index of the protective power of the serum. It is interesting, as a test of the effect of virus inoculation on protection test results, to compare the means of the average survival time of mice with pre- and postinoculation sera from the same animal. This has been done in table 6. This table is based on the experiments reported on previously by Bugher *et al.* (1941), as well as on those covered in the present paper, in order to include as large a number of animals as possible. The 5 Saimiri monkeys are those listed in detail in table 1 of Bates (1944). The "rhesus controls" are based on 22 tests, each with pools of normal serum and dilutions (1:4 to 1:40) of immune pools used in the protection tests in which marsupial sera were run.

Even if a certain number of preinoculation sera of the marsupials were positive because of exposure to yellow fever in the wild state, the postinoculation sera of these animals should continue to be positive, so that the mean average survival time of postinoculation sera as a whole should be dis-

tinctly greater than the preinoculation survival time. This is true with the rhesus controls, with *Saimiri sciureus*, and with *Metachirus nudicaudatus*.

The difference with *Didelphis marsupialis* is so slight that it might well be considered as chance, and the postinoculation sera of *Metachirops opossum* actually show a slightly lower survival time than the preinoculation sera!

TABLE 6

Average survival time of mice used in intracerebral protection tests of pre- and postinoculation sera from the same animals

SPECIES	NO. ANIMALS	MEAN OF AVERAGE SURVIVAL TIME OF PROTECTION TESTS		
		pre-inoculation	post-inoculation	Difference
Rhesus controls*.....	22	5.40	9.19	+3.79
<i>Saimiri sciureus</i> †.....	5	5.18	7.94	+2.76
<i>Metachirus nudicaudatus</i> ‡.....	24	5.87	7.60	+1.73
<i>Didelphis marsupialis</i> §..	64	5.50	5.79	+0.29
<i>Metachirops opossum</i> ¶..	35	6.55	6.44	-0.11

\* Control sera on protection tests: the postinoculation sera are dilutions from 1:4 to 1:40 of immune pools.

† Histories detailed in table 1 of Bates (1944).

‡ Twenty animals with complete histories from present studies and 4 from experiments made by Dr. J. C. Bugher.

§ Data from experiments by Dr. J. C. Bugher: means of all animals on which complete histories were available.

¶ Fourteen animals from experiments reported in the present paper and 21 animals from experiments made by Dr. J. C. Bugher.

Specific examples of erratic behavior can readily be found by inspecting the results summarized in tables 2 and 3 (*Metachirus*) and table 4 (*Metachirops*). It is difficult to convince oneself that some of the unexpected results with the brown masked opossum may not be due to mistakes. The results with animals 176 and 177, for instance, would seem perfectly clear if one assumed that the animals were switched before the postinoculation bleeding. In other instances, however, we have been unable to explain the results by assuming a mistake with either the animals or the sera, and it seems clear that "false positive" reactions occa-

sionally occur in the brown masked opossum (No. 183 (2) in table 2 and No. 131 in table 3).

These "false results" are exceptional with the brown masked opossum, and a much larger series of experiments would be necessary before their significance could be evaluated. An inspection of the results with the grey masked opossums (table 4), however, shows that all results seem to be completely random. The only animal that showed circulating virus (No. 135) had a preinoculation test that would normally be considered "positive," and animal No. 101 shows a clearly positive preinoculation test and an apparently negative postinoculation test—the two made in the same protection test run. A similar result was obtained on a repeat test with the preinoculation serum. The protection test data obtained by Dr. Bugher with the grey masked opossum show a very similar distribution to the data summarized in table 4. In all of Dr. Bugher's records on this species, 16 of 44 wild sera were interpreted as "positive;" in our recent tests, 9 of 28 sera were so interpreted. This gives a 36 per cent "positive" result in the first series, and a 32 per cent "positive" result in the second series. The 3 animals from which Dr. Bugher recovered virus all showed clearly "negative" preinoculation protection tests, but this might well have been due to chance, just as it is surely a chance result that the only animal from which we secured virus showed a "positive" preinoculation test. The striking similarity between the preinoculation and postinoculation tests of the fairly large series of grey masked opossums averaged in table 6 would seem surely to mean that the protection test result is not related to exposure to yellow fever virus in the case of this particular species.

The interesting thing is the sharp difference in behavior, both with regard to circulation of virus and protective power of serum, between the brown and the grey masked opossums. It seems to us that the virucidal properties shown by the sera of the grey masked opossums are probably largely non-specific, and that the specific response in the brown species is confused by occasional non-specific reactions. A similar situation has been reported by Findlay *et al.* (1936) with sheep sera, and by Bugher (1940) with sera of various animals. One cannot be sure at present to what extent these anomalous protection test results with marsupials are properties of the sera, and to what extent properties of the type of test used. The

range of possible differences in protection test results with the same serum, depending on the type of test used, is nicely shown in a recent paper by Whitman (1943). The subject is surely worth more detailed investigation.

#### *Toxicity of Serum for White Mice*

In a previous paper (Bates, in press) the toxicity of Saimiri monkey serum for white mice is discussed. The same phenomenon is shown to a varying degree by sera of various species of marsupials. Mortality caused by inoculations with pure serum and by tenfold dilutions has been summarized in table 7; the "No. killed" is based on mice found dead 24 hours after inoculation, although the toxic action of the serum is usually

TABLE 7  
*Toxicity of marsupial sera for mice*

SPECIES	PURE SERUM			DILUTION 1:10		
	No. inoculated	No. killed	Per cent	No. inoculated	No. killed	Per cent
<i>Metachirus nudicaudatus</i> ...	426	9	2.1	426	6	1.4
<i>Metachirops opossum</i> .....	258	104	40.3	258	6	2.3
<i>Didelphis marsupialis</i> .....	222	86	38.7	222	4	1.8
<i>Caluromys laniger</i> .....	72	3	4.2	72	3	4.2
( <i>Saimiri sciureus</i> ).....	528	308	58.3	708	23	3.2

immediate. The tenfold dilutions show about the normal mouse mortality from injury due to the intracerebral inoculation itself, and serve as a control on the mortality caused by pure serum.

The interesting thing about this table is the sharp difference between the serum of the brown masked opossum (*Metachirus*) and the grey species (*Metachirops*). In this respect, as well as with regard to reaction to the virus of yellow fever, the 2 species show very different serological properties. There seems to be no relation between this toxic action and yellow fever: *Metachirops*, resistant to yellow fever infection, has serum toxic for mice, as does *Saimiri*, a species highly susceptible to yellow fever infection. *Metachirus*, a relatively susceptible animal, shows little or no toxic serum action. It is interesting that *Metachirops*, with respect to toxicity of serum, sus-

ceptibility to yellow fever, and protection test reaction, seems to be more similar to *Didelphis* than it is to *Metachirus*.

#### DISCUSSION

If we combine the experiments reported by Bugher *et al.* (1941) and those reported in the present paper, we get the following rates for recovery of virus in animals inoculated with known virus:

Brown masked opossum: 34 of 47 animals = 72 per cent.

Grey masked opossum: 4 of 43 animals = 9 per cent.

Animals that have been bled daily have often shown circulating virus on only one bleeding, so it is likely that some cases of circulating virus have been overlooked because the animal was not bled on the proper day. Nevertheless, the figures are comparable, in so far as both species were bled with about the same regularity.

With the brown masked opossum, several animals that have been inoculated with small virus doses and that have not shown circulating virus have subsequently circulated virus after a second inoculation. This same behavior has been found in the case of 2 common opossums (*Didelphis marsupialis*). This would seem to show that the first inoculation was completely non-infective. We have on several occasions tried reinoculating animals that have circulated virus once, but in no case have we been able to demonstrate circulating virus a second time, though this would seem theoretically possible. It seems at least clear that mere inoculation with an appreciable virus dose (as measured in terms of mouse m.l.d.) does not necessarily result in immunity to subsequent infection in either *Metachirus* or *Didelphis*, and this in itself indicates that susceptibility and resistance to yellow fever virus in these animals differ strikingly from that observed in primates.

It also seems curious that the serial passage of virus through 10 brown masked opossums resulted in no demonstrable increase in virulence or infectiousness for that species or for the related grey masked opossum. The strain of virus used seemed as precariously adapted to the marsupial host at the end of the experiment as at the beginning. One is led to suspect that this serial passage experiment was a mere *tour de force*: a laboratory phenomenon impossible to interpret in terms of possible relationships between virus and marsupials under natural conditions.



It seems to the author that it is impossible, in the present stage of our knowledge, to evaluate the results of any of these marsupial experiments in terms of the possible epidemiology of jungle yellow fever. Several unsuccessful attempts have been made to infect mosquitoes on marsupials, chiefly using *Haemagogus capricornii*, the species presumed from field evidence to be the chief local vector. These failures, however, cannot be given much weight because, while it has been possible to infect this mosquito by allowing it to feed on Saimiri monkeys, transmission under laboratory conditions has been highly irregular even in Saimiri experiments. We have secured transmission with *Haemagogus* infected on Saimiri monkeys only when the mosquitoes have fed on monkeys circulating virus with a titer in excess of 1:100,000. We have in no case found virus in circulation in marsupials with titers appreciably in excess of 1:1000 (always judged by mouse inoculation) and under these circumstances infection on marsupials would seem, *prima facie*, unlikely. Yet it is very possible that some important factor in the transmission mechanism has been overlooked, and judgment should be reserved until we have a better understanding of the conditions governing the infection of mosquitoes like *Haemagogus capricornii* under laboratory conditions.

#### SUMMARY

1. The virus of a Colombian strain of yellow fever was maintained in the brown masked opossum (*Metachirus nudicaudatus*) for 10 consecutive passes by the intramuscular inoculation of serum.

2. There was no apparent increase in virulence or infectiousness of the virus for the brown masked opossum or for the related grey masked opossum in the course of these passes. The virus seemed equally infectious for Saimiri monkeys both before and after serial passage in the brown masked opossum.

3. The grey masked opossum (*Metachirops opossum*) was found to be resistant to infection with the strains of yellow fever virus tested; circulating virus was recovered from only 1 of 28 animals.

4. Brown masked opossums which failed to show circulating virus after inoculation with small but demonstrable doses of virus sometimes circulated virus after a 2nd inoculation with the same or different strains of virus. Animals that had circu-

lated virus once failed to show a 2nd circulation of virus on reinoculation.

5. Antibody response, as measured by the intracerebral protection test, was usually regular in the brown masked opossum, but a few sera seemed to give "false reactions" and a somewhat larger number of sera failed to show protective power even though taken from animals that had circulated virus.

6. The results of the intracerebral protection tests with sera of the grey masked opossum seemed to be completely unrelated to exposure of the animals to the virus of yellow fever.

7. Serum of the grey masked opossum was found to be highly toxic for mice on intracerebral inoculation, killing about 40 per cent of the mice; serum of the brown masked opossum did not show this toxic property.

8. The evidence available with regard to the possible role of these marsupials in the epidemiology of jungle yellow fever is inconclusive.

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# THE ANOPHELES OF PANAMA WITH SPECIAL REFERENCE TO HAND LENS IDENTIFICATION AND NOTES ON COLLECTING AND CARE OF SPECIMENS

THE LATE C. P. BAXTER, LIEUT. COLONEL, M.C., USA, AND JAMES ZETEK, ENTOMOLOGIST,  
U. S. DEPT. AGRICULTURE

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## PART I

Medical personnel accompanying the Armed Forces in the field, at home, and scattered widely over the world, make this an unprecedented opportunity for the collection and study of mosquitoes.

Not all mosquitoes transmit disease, and not all *Anopheles* transmit malaria. If only from an economic point of view, the impossibility of eliminating all forms of mosquito life in or about militarized areas, isolated outposts, airfields and temporary bivouacs can readily be seen.

Accurate knowledge of the habits of mosquitoes, as to the conditions under which they breed, their range and hours of flight, their day time hideouts, their preference for animal versus human blood, whether or not they enter houses to feed, etc., and, above all, whether or not they are capable of transmitting disease, is, therefore, of economic as well as medical importance, to enable Medical Officers to successfully combat their menace to the health and efficiency of the Military and Naval Forces.

The above is equally applicable to any enlightened civil government which works with, or follows, Field Forces.

Yellow fever and malaria have changed the course of history and may well do so again.

There are about 1600 known species of mosquitoes distributed over most of the world, of which nearly 200 belong to the genus *Anopheles*, and yet, at this time, only about one tenth of these *Anopheles* are recognized as important vectors of human malaria.

Troop concentrations and careful entomological studies in new Theatres of Operation will undoubtedly incriminate new or hitherto unsuspected species.

Of all the species, *A. gambiae*, of Equatorial Africa, is the greatest enemy of man, not only because of her persistence in hunting human bait and her ability to transmit the three most important types of human malaria, but because of her high degree of infectivity.

About 1930, *Anopheles gambiae* was accidentally introduced in to the New World by French gunboat or plane. Although originally confined to one Brazilian valley, it became so important that, during the first six months of 1938, it alone caused the most severe epidemic of malaria ever occurring in the Americas, with 100,000 cases and 14,000 deaths in that half year (1).

*Anopheles* mosquitoes breed as far north as Southern Finland, menace the personnel of ships in malarial ports, as well as entire populations, and annually cause untold disability among millions.

This paper, then, is written for the man in the field, equipped only with a hand lens, reference data in compact form and a few rugged articles of collecting paraphernalia, in order that he may obtain specimens of eggs, larvae and adults from the area in which he is operating, and be able to make a working identification.

Only when their identification has been made, habits, preferences and disease bearing possibilities studied, from reference data of known species, or carefully worked out from study of new species, can the problem be solved.

### *Life history and habits*

*Adults.* After emerging from the pupal case, the newly metamorphosed adult (imago) rests on its empty floating pupal case for a few moments, preening and exercising its wings, after which it flies away to a nearby hideout in daylight, or to the nuptial flight if at night.

Shortly after this swarming, the males depart for the vegetation upon which they entirely exist, while the gravid females (possibly after a few vegetable juice meals) seek a blood meal, according to their specific inherited habits.

Non-infectious at this stage, it is only after she has bitten an individual infected with some mosquito borne disease that she becomes infectious.

Unfortunately, the gravid female demands more than one blood meal and, because she may remain

# CHART 1

ADULTS: Table of preferences of Panama Anopheles

	ENTERS HOUSES TO FEED	MAINLY ON ATLANTIC OR PACIFIC SIDE	MONTHS OF YEAR	BLOOD PREFERENCE HUMAN (H) OR ANIMAL (A)	INFECTED IN NATURE	COMMON OCCAS- IONAL, RARE
1. ALBIMANUS.....	Yes	A & P	All	H > A	Yes	C
2. AQUASALIS.....	Yes	A > P	All	A > H	Yes?	C
3. STRODEI.....	Yes	P > A	All	A > H	Yes?	O
4. TRIANNULATUS.....	No	Fresh water	All	A > H	No?	R
5. ANOMALOPHYLLUS.....		Inland				R
6. OSWALDOI.....	Rare	Mainly A	8-11	A > H	?	O
7. ALBITARSIS.....	Rare	A	All	A	Yes	C
8. ARGYRITARSIS.....	Rare	A & P	All, mainly 1 & 2	A > H	?	C
9. KOMPI.....	?	A	12-2	H	?	R
10. PARAPUNCTIPENNIS.....		Inland				R-A high- land mosquito
11. PSEUDOPUNCTIPENNIS.....	Rare?	A & P	All, mainly 1-3	H? A	Yes?	C
12. EISENI.....	Rare	A > P	Mainly 9-3	A	?	C
13. VESTITIPENNIS.....	Yes	Inland		H		R
14. APICIMACULA.....	Rare	A & P	All, mainly 4-2	A > H	?	O
15. PUNCTIMACULA.....	Yes	A & P	All	H or A	Yes	C
16. NEOMACULIPALPUS.....	?	A & P	All	?	?	O
17. NEIVI.....	Rare	A	7-2	H	?	O
18. BATHANUS.....	No	Mainly A	All, mainly 11 & 12	A > H	?	R

# CHART 2

LARVA: Table of preferences of Panama Anopheles

	WATER				MAINLY ON ATLANTIC OR PACIFIC SIDE OF INLAND	MONTH OF YEAR
	Fresh or brackish	Clean or dirty	Sunny or dark	Still or running		
1. ALBIMANUS.....	F or B	C	S	S or R	A & P	All
2. AQUASALIS.....	B > F		D > S	Mainly tidal	A > P	All
3. STRODEI.....	F	C	S > D	S	P > A	All
4. TRIANNULATUS.....	F	C	S	S	I	All
With aquatic vegetation						
5. ANOMALOPHYLLUS.....	F	C	D	R > S	I & A	
6. OSWALDOI.....	F	C	D	S	Mainly A	8-11
7. ALBITARSIS.....	F	C	S	S	A	All
8. ARGYRITARSIS.....	F	C	S > D	R > S	A & P	All, mainly 1 & 2
9. KOMPI.....	F	C	D	S & R	A	12-2
10. PARAPUNCTIPENNIS.....	F	C		R > S	I	
11. PSEUDOPUNCTIPENNIS.....	F	C	S	S > R	A & P	All, mainly 1-3
12. EISENI.....	F	C	D	S > R	A > P	Mainly 9-3
13. VESTITIPENNIS.....	F	D	D	S	A & I	
14. APICIMACULA.....	F	C	D	S > R	A & P	All, mainly 4-2
15. PUNCTIMACULA.....	F	C	D	S	A & P	All
16. NEOMACULIPALPUS.....	F	D	S	S	A & P	All
17. NEIVAI.....	F	Only in wild pineapple			A	7-2
18. BATHANUS.....	F	C	S or D	S or R	Mainly A A	All, mainly 11 & 12

infectious for days or months, she remains a menace.

The propensity of the males to avoid humans, and for many species of females to seek humans, explains why, in an "honest catch" of adults, the latter far outnumber the former, unless the catch was made at or very near the breeding waters.

The gravid female deposits her eggs according to instinctive habits of her particular species (see chart 2), which make the Sanitary Inspector's work simpler, provided he is familiar with, or has handy access to data on these important points.

Having deposited her eggs, singly, as in *Anopheles* and *Aedes*, or in honeycomblike rafts, as in *Culex*; in water, mud or, in some species just above the water line, she flies away for further impregnation and blood meals.

Her range of flight varies from a few hundred yards to several miles, if wind borne (2).

Practically all species seek dark day time hiding places except, of course, those who naturally inhabit deeply shaded areas in the Rain Forests, and these may become annoying day-time feeders.

Mosquitoes are attracted to lights and light colors, hence the fact that white men and animals are more attractive bait than their darker fellows.

Body surface area also attracts, thus a white horse attracts more than most other animals, a fact that has been used by one of us to advantage, in placing our pack train between the town and our troops in bivouac.

Conversely, smoke and smudges are avoided, as are certain essential oils and chemicals.

Mosquitoes can, and do, habitually withstand the rigors of subarctic temperatures. They cannot, however, survive heat of over 120 degrees Fahrenheit, so are absent in certain areas during the summer.

*Eggs, larvae, pupae.* After a few days, depending upon favorable circumstances, such as warm weather, the eggs hatch, producing the first stage of larval life.

These tiny larvae spend most of their time just under the surface of the water, or in the case of *Mansonia* and some other genera, are more or less permanently attached to the roots of aquatic vegetation. If disturbed, the surface breeders dive or scurry away.

They moult several times, each time shedding their skin. In warm weather, these moultings may take place in three to five days, when partial metamorphosis takes place, giving origin to the pupal stage.

The pupal stage may last from 24 to 48 hours. These pupae likewise spend most of their time at the surface of the water. When disturbed, they "tumble" away in a series of somersaults or rapid, active, jerky motions.

### *Collection of specimens*

It is not intended to discuss here all the methods in use, but merely those that may be used under field conditions. Inasmuch as adults, eggs, larvae and pupae are desired for study, there are certain items of collecting equipment necessary for the adult group, and for the other stages.

When going on a mosquito hunt, proper equipment should be taken into the field. Some suitable carrying case is essential, and this may be in the form of a canvas bag containing pockets, in which bottles and other equipment may be fitted.

A leather German cartridge case makes an excellent container, having, as it does, three separate pockets, each 3" by 3" by 3", as shown in photograph. In the field, this is best carried on the web pistol belt, and should contain items for the collection, examination, identification and transportation of adults, eggs, larvae and pupae.

In any event, it should contain corked vials, at least  $\frac{3}{4}$ " in diameter and a set of pill boxes already prepared. These pill boxes should have a small amount of powdered naphthalene and paradichlorobenzene in the bottom, with a tight fitting disc of blotting paper perforated in several places with a needle. This combination protects against psocids and other predacious insects, and also helps materially to keep out mould. If paradichlorobenzene is not readily obtainable, then a concentrated solution of naphthalene in beachwood creosote can be used, a drop of this on the bottom of the pill box, and over this either a disc of blotting paper or a disc of sheet cotton.

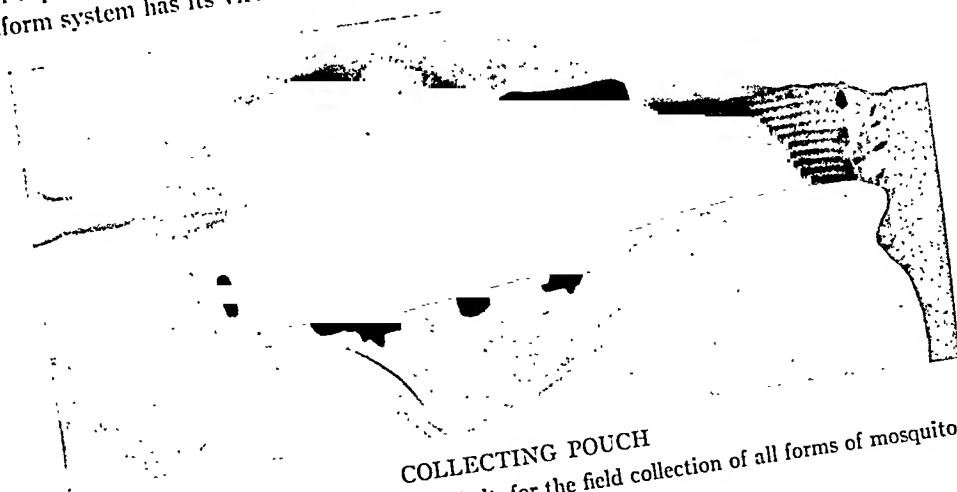
The mosquitoes in the killing tube should be allowed to slide into the pill box, not too many to a layer, then a disc of sheet cotton, or soft tissue paper, then more mosquitoes, another disc, and so on. The important thing to remember is *not* to handle the specimens with the fingers, and to take every precaution not to rub off any of the scales. The pill box should not be packed down, but also the mosquitoes must not be so loose that they can move about. Some taxonomists insist in even greater care, which merely emphasizes that a rubbed, or poorly-cared for specimen is worthless.

Each pill box must be identified with a number

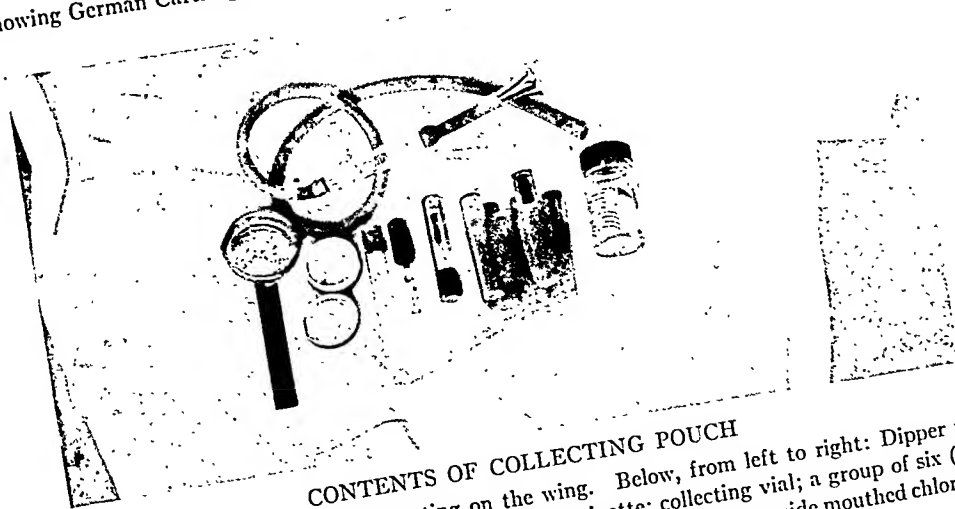
and data, this data brief because of the limited space on the lid of the pill box,—it should give at least the exact locality, date collected, and name of the collector. More detailed entries should be kept in a pocket-sized note book, or on cards, and a uniform system has its virtues. Data as to

and broken off so as to give an opening about the full size of the dropper. This tip should be glazed in a flame so that larvae or pupae passing thru it will not be injured.

The kit should also contain a small vial of chloroform, and a small dipper or unbreakable



COLLECTING POUCH  
Showing German Cartridge case on web belt, for the field collection of all forms of mosquito life



CONTENTS OF COLLECTING POUCH  
Above: Mouth suction apparatus for collecting on the wing. Below, from left to right: Dipper with folding handle; prepared pill box; vial of chloroform; wide mouthed pipette; collecting vial; a group of six (6) collecting vials, protected by a holder made from metal window screening, and a heavy glass, wide mouthed chloroform killing tube with screw cap.

date, time of day, place, building or hutment should be recorded, as well as notations regarding wind direction and velocity, character of the weather, etc. Of special importance are notations as to whether specimens were feeding on humans or animals.

The kit should contain a pipette, made from an ordinary medicine dropper, the tip file-marked

saucer, preferably white, with which to scoop up larvae. For a compact kit, a dipper made from the bottom of a metal pill box, as shown, 2" in diameter and  $\frac{1}{2}$ " deep, is satisfactory, provided a pouring lip is shaped into the edge, and a small piece of steel spring, possibly 6" long, soldered to its base, as shown in the illustration, forming a semi-flexible handle to reduce space in packing.

A set of ruled cards 3" by 5", should be included, and a flashlight carried, for searching out mosquitoes in their hideaways. A hand lens of preferably 10 diameters magnification must be carried if identification is attempted in the field, plus a compact, durable chloroform killing tube.

*Adults.* Elaborate traps utilizing electric lights and suction fans, as well as carbon dioxide (in the form of dried ice), or various combinations of these, have been described. For the purposes of this field paper, none is practicable.

*Trapping.* An effective and simple net trap may be made by suspending one mosquito bed net over another, with a space of a few inches between the two, at the top and sides. The inner net is placed over the bed of a man, in the regular manner, properly tucked in and made mosquito proof; the outer net is then loosely placed over the inner, but openings are left about its base. On waking, or just before dawn, the outer net is tucked under the bedding, when mosquitoes, which have been attracted by the human bait, may be killed by spray or caught in a chloroform tube or stupefied by tobacco smoke and captured. Another method is to suspend a mosquito net over an animal; a horse, preferably white, offers excellent bait. The net should have weights, sewn into its lower free edge or a purse string fastened in its hem. Numerous mosquitoes may be caught in this manner after the suspended net has been rather suddenly dropped over the animal.

Other methods of value include a dark damp box or keg. An empty nail keg placed on its side and well covered with wet gunny sacking (part of which falls over the top of the opening) makes a natural day hiding place.

A trap may be made by fitting a cone shaped piece of mosquito wire in a window screen or bed net. The apex of the cone is opened about one-half inch and a glass jar fitted tightly over the small end thus leaving an entry way and a baffle to prevent exit; not unlike a cage rat trap.

*Hand catching. Chloroform killing tube.* The well known chloroform killing tube widely used for many years, is prepared as follows,—A wide mouthed test tube is filled to a depth of about 1", with a tightly wadded mass of absorbent cotton, which is saturated, about once each week, with commercial chloroform. About midway between this mass of wet cotton and the mouth of the tube, a lightly packed bit of cotton is placed, it being large enough to remain in place. A piece of leather

or paper cut the diameter of the tube is placed over this cotton and the tube tightly corked.

This tube, uncorked, is placed, with an initial slow movement, followed by a final rapid thrust, over a resting adult, who is rapidly killed therein. Care should be taken not to scare away the insect by casting a shadow or blowing smoke over it. Mosquitoes should be left in the killing tube only long enough to kill them.

To collect males, it is necessary to sweep grass in their breeding places, with a net made of mosquito netting attached to a frame, not unlike a trout net. The circular opening should be at least 18" in diameter and the net should be cone shaped and about 2' long. Males are only caught near their breeding places.

*Suction apparatus.* Females are normally caught around habitations, and upon animals and man, and it is necessary to collect them, if on the wing, with a suction tube. This consists of a small glass bottle with a funnel shaped opening in one end and an attached rubber tubing at the other end. A small bit of mosquito netting is put over the exit to which the rubber tubing is fastened. Two feet of rubber tubing, attached to a small mouthpiece, is used, and by mouth suction at arms length, mosquitoes in flight are drawn into the funnel shaped entrance of the bottle where they may be killed with chloroform.

Cyanide used to kill, is inferior to chloroform, as it bleaches if specimens are left in it for too long.

The best time for collecting indoors is at, or just after dawn, and at, or just after dusk. During the rest of the day the mosquitoes are well secluded and difficult to find. During the hours indicated they are nearly all at the screens.

Since mosquito catching is a very important measure in the reduction of malaria, too much care can not be taken in the selection of good mosquito catchers, and enough additional men should be trained, from time to time, so that if more catchers are required, or if they are unavailable for any reason, there will always be others ready to step into the work. The eyes of all mosquito catchers should be examined, both regarding sight and color perception.

Sometimes mosquito catchers become lazy or otherwise indisposed to do their work honestly and well. Such men sometimes breed out mosquitoes in order to have a supply on which to draw, or else they retain mosquitoes which the Sanitary Inspector has already examined, to



submit them another day. Extremely dry specimens turned in by a mosquito catcher always should create a suspicion, and if, in the case of *Anopheles*, there are males present in the catch, this too, should require investigation. The best procedure in each case is to assume nothing unusual was noted, and to observe quietly the guilty collector's acts.

Another source of valuable material and information is collecting in the field at and after dusk. Often this requires only quiet and patience, because if *Anopheles* are present, they will soon locate the bait. Two men are better than one because one of them can collect from the back of the other.

*Eggs, larvae, and pupae.* No general rules can be given as to where to search for eggs, larvae, and pupae. A sane guide is to overlook no body of water, however small, and especially to search for small pockets of water, natural or artificial, that are protected by tall grass; search in discarded tins, pontoons, cayucas or coconut shells, as well as in the bases of palm leaves, or in water held between the leaves of bromelias, heliconias, etc., and even the stems of certain aquatic vegetation, as well as pot-holes and tree holes.

The only exception is water recently oiled.

A precaution that is not often heeded and which, if neglected, will yield negative results, is not to allow one's shadow to pass over the water, or even to tread, other than lightly, when approaching a suspected water collection. If these points are neglected, larvae and pupae generally dive to the bottom, where they may remain several minutes.

*Eggs.* These are at best difficult to detect. A convenience is to strain surface water thru a tiny white flannel strainer from which eggs (as well as larvae and pupae) may be removed with the pipette, and placed in vials containing water in which they were found.

*Larvae and pupae.* These should be scooped up and strained, or removed from scoop by pipette, or poured directly into jars. A serviceable scoop is an ordinary white enamel dipper, cup or saucer. If a portion of the white lining is covered with black enamel paint we have found that, under certain light conditions, and colors of larvae, they can be more readily detected. The first instar (moult) may be only 1 millimeter long and nearly transparent.

In searching out pot-holes and tree holes, a long piece of rubber tubing may be connected to the open end of the pipette.

Care should be taken to prevent the specimens from much jarring enroute, and from exposure to intense light and heat. Specimens from each area, separately bottled, should be labeled, and cross references made to notes.

*A floating trap.* An excellent type of trap for use in the dry season, at least, has been used by one of us during the past two years. It can be used in lakes, tide water, streams and ditches. In lakes and tide water, it has some value locally the year round. Torrential rains, however, make it of no value in streams and ditches during the rainy season, because of its capsizing, being washed away, and fouled with debris.

Cheaply and rapidly constructed, it consists essentially of a wooden trough open at both ends, not unlike a carpenter's home made miter box. Ours have been made of  $\frac{1}{2}$ " boards, 6" wide, and never more than 18" long. Its upstream end (or upwind end when used in lakes or tide water) has a series of 3 wire baffles 6" square; the first being cut from a piece of chicken wire of approximately 1" mesh, and tacked to one end of the box.

The second baffle should have approximately  $\frac{3}{4}$ " openings and is fitted into the box about 2" from the first one. The third baffle, or strainer, is made by pulling enough strands from an ordinary piece of window screening, cut 6" square, to make a mesh with openings about  $\frac{1}{4}$ ", and this is fitted about 2" from the preceding one.

The object of these strainers is to prevent floating debris from entering, and to allow eggs, larvae and pupae to pass through, where they are finally caught in a piece of ordinary cotton sheeting which is fastened to the lower end of the box.

This floating trap, sufficiently weighted with rocks or pebbles to remain half submerged, is anchored by a stout cord attached to the wire mesh end, where, if in a lake or tide water it will shift with the wind or tide.

It should be inspected at least twice each week, when debris should be removed, specimens collected, and its ballast altered if necessary.

### Care of specimens

*Adults.* The important points are: first—that the specimen should be handled with great care and as little as possible, and, second—that, particularly in the Tropics, they may deteriorate rapidly, almost over night, unless protected from mites and moulds.

The scales and tarsal tips, essential for differential diagnosis, are easily damaged or broken off

completely. Mosquito catchers must, therefore, be warned never to touch the insects, but to slide them gently into a prepared pill box and to distribute them evenly therein. The catch should be emptied, when passing from one location to another, into a box, but should not be emptied while exposed to a wind or rain.

After returning to a Base Camp these should be placed in a large glass jar, with a tight fitting top. It is assumed that the pill boxes have been treated as already explained, and hence, if care was taken in handling the adults, these should be in good condition when stored in the jar. It is well to fumigate the jar of pill boxes containing the adults, at least once a month. This fumigation is simple, as all that is necessary is to pour some carbon-bisulphide into the jar, preferably upon a small dish placed on top of the pill boxes, and to have the lid closed tightly. The dosage is about 2 cubic centimeters of carbon-bisulphide per cubic foot of space, a few drops per quart or pint jar; and the specimens should remain in fumigation at least 24 hours. An even simpler method is to put into each jar containing pill-box material about a tablespoonful of powdered naphthalene and para-dichlorobenzene (50-50), renewing this when depleted.

For permanent preservation carefully selected specimens, freshly killed, with wings in full spread, should be impaled on tiny entomological pins (minuten nadeln). Lacking such pins, very small sharp cactus, black-palm or other thorns 1 cm. long, may be used to puncture the thorax thru and thru. The spread wings are then carefully and delicately pinned in position on a flat cork surface (a bottle cork is large enough) and the whole placed in a jar of carbon-bisulphide fumes, and stored in a dark place for several days, when the wing holding pins may be removed.

The next step is to cut a 3 millimeter cork cube; this is slightly perforated with a large pin and the blunt base of the thorax piercing pin, or thorn, firmly inserted. This cork cube is perforated with a large common pin, the point of which is then imbedded in a larger cork, fitted to a test tube of about 1 cm. diameter. A drop of carbon-bisulphide is then placed in the test tube and the insect on the "double pin-cork set up" is put in, the test tube tightly corked, and thus it is permanently protected and available for hand lens study from all angles.

If microscopical slides and cover slips are available at a Base Camp, specimens can be saved for

later microscopical study. Adults should be cleared in oil of clove, wings spread, partially dried, and mounted on their sides in a drop of Canada balsam, under a cover slip.

*Eggs, larvae and pupae.* These may be preserved, following the usual procedure for making balsam mounts.

Except for differentiating the eggs of *Anopheles*, *Aedes* and *Culex* from one another, it is believed that nothing more can be accomplished with a hand lens. However, these forms may, and frequently should, be bred out, being sure to use either the water in which they were found or, lacking sufficient quantity of this, rain water (not roof drainage) collected in clean containers, or distilled water; preferably in the order named. Ordinary clean tumblers or preserve jars of various sizes should be on hand. After putting specimens in, these should be covered with a layer of gauze tied firmly in place.

Properly marked for future identification, these should be stored in any safe part of a screened building or tent. These specimens must not be exposed to the direct rays of the sun. If so they may be killed by heat. Care should be taken to provide resting places, such as clean stones or chips of floating wood, in order that adults will not be forced to cling to the glass until they become exhausted, and fall into the water, where they drown.

When hatched, the imagoes may be killed (after they are "hardened" and fully colored) in batches with chloroform, or stupefied by tobacco smoke, or allowed to fly into a larger breeding cage, in which, if food in the form of fresh yeast is provided, they may breed if given an occasional blood meal from an animal or human.

If human biting is practiced, only one individual should be allowed to provide blood for any one cage of insects. Otherwise malaria may be spread as a laboratory infection. Such a transfer cage should only be permitted in a room or outbuilding screened off from adjacent habitations, as a sanitary precaution.

#### *Study of specimens*

*Adults.* A rapid general examination should first be made, noting sex and tribe; scanning the thorax and abdomen for distinctive markings; also wings, head ornaments and hind legs.

In the case of *Anopheles*, a female will give more information, when examined with a hand lens only, than a male and, in insects caught in nature,

the females will ordinarily outnumber the males 50 or 100 to 1.

Note first the hind legs, checking coxa to prove that, in an apparent tangle of legs, the hind legs are, in fact, under observation. Note in turn femur, tibia, the first (the longest) tarsal segment, and carefully check to make certain that there are not only five tarsal segments present, but a tarsal claw as well.

Always and invariably, not only examine against a white, as well as a black background, but by direct and transmitted light. A broad black and white band of paper fastened, thimble fashion, (or a black and white leather finger stall used by one of us), over the tip of the 4th finger, is a great convenience, as examination can proceed against black or white background at will, by a slight movement of the finger holding the contrast background.

Note general coloration and markings of each joint, checking carefully against plate. Note especially spots, bands and rings.

In the *Nyssorhynchus* group, note the black-white ratio on the second hind tarsus in great detail, and compare it with its fellow on the opposite side, inasmuch as the percentage of basal black to apical white is of diagnostic importance in many cases. Note black-white ratio and location on fifth tarsus. Note markings and coloration of femur and tibia. If spotted or speckled, note ground color; note color, amount, location and size of spots.

Male and female hind legs are identical in markings.

The female palps offer valuable clues, the five sections being examined in turn. Note the amount and exact location of white or light scales, especially as to whether white scales are at the base or apex of palp segments.

In the wings, male and female markings are identical, as are the markings of the legs. Note the size, exact distribution and the color of the wing vein scales and spots. Note especially the coloration of the fringe where, in general, a dark tipped vein is followed by a dark fringe patch and vice versa. Colors other than black or white are important.

At each stage of examination turn to the corresponding hind leg, female palp, or wing plate for comparison with the drawings. It will be noted that hind leg markings place the mosquito under observation in one of several groups, which are so arranged; white tarsal segments, mainly black

legs; speckled legs, banded legs; or variously marked.

Proceed with the study, using a hand lens only, and continue comparison with the prints, but under "Field Notes" check additional vital data, essential to differential diagnosis in some few cases.

The proportion of black and white on the hind legs of a given species in the same area is generally fairly constant. But where a species has a wide distribution, specimens from the extremes of this range may, and in some species do, show variations; in some cases this is considerable. The same applies to the black and white on the palps. It also applies to the wings, where not infrequently aberrations are found, or the two wings on the same individual do not coincide. Nevertheless, in a relatively small area, such as Panama, confusion lessens as one gets more familiar with the species, and familiarity comes thru seeing large series from as many locations as possible. Thus one gets to recognize *albimanus*, *argyritarsis*, etc. quite readily. Very early in the study one has no difficulty in recognizing the *Nyssorhynchus* group which contains the principal vectors of malaria. It is unfortunate that there are no uniformly definite, constant, clear-cut characteristics visible thru a hand lens which will positively differentiate every species of *Anopheles*.

Not everyone has the time to study genitalia, and too often time is a limiting factor; and, to be able to properly study the male genitalia, training and experience are needed. This does not mean that the males should be neglected. There are specialists who will welcome properly preserved males, as well as females and larvae. Instructions, written for the man in the field equipped only with a hand lens, should not include any description of the male genitalia.

*Eggs, larvae and pupae.* Eggs of the various species of *Anopheles* cannot, as stated above, be distinguished one from another with a hand lens. Whereas the eggs of *Culex* are always laid in rafts, standing upright, cemented one to another, those of *Aedes* and *Anopheles* are always laid separately. *Aedes* eggs typically remain floating alone, whereas those of all the *Anopheles* generally form geometric designs, as they float on the surface. Each *Anopheles* egg has a "float" on either side, which resembles a tiny, light colored, semi-lunar body.

Having determined that the larva under investigation is an *Anopheles* and not of the genus *Uranolenia* (which although resting parallel to the surface of the water, possesses a long breathing

tube, absent in all *Anopheles* larvae), examination may proceed, even in the Field, with a hand lens, using the method described by one of us (3) which is rapid and satisfactory. It can be used, however, only if the species of *Anopheles* of the region are known, or determined by exhaustive breeding tests.

In addition to a hand lens, preferably of 10 power, all that is required is any small receptacle, such as a beer cap, and a few drops of milk.

The larvae are examined alive, after being carefully placed in the milk with the medicine dropper, where they come to the surface and remain very quiet. All that is seen are the palmate hairs, the antennae and the moving mouth brushes. The palmate hairs stand out in bold relief against the white background and permit a prompt "working diagnosis" to be made, in the Interior of Panama at least, as to the presence of members of the *tarsimaculatus* series. Inasmuch as this series contains Panama's most important vectors of malaria, it is advisable for troops to avoid areas where larvae showing palmate hairs, on segments 1 to 7, both inclusive, are detected.

Similar data can probably be worked out in other areas.

Equipped only with a hand lens, no attempt should be made to differentiate species from the pupal stage.

## PART II. THE ANOPHELES OF PANAMA

### 1. ANOPHELES (NYSSORHYNCHUS) ALBIMANUS Wiedemann 1821

Important synonyms:

*Anopheles cubensis* Agramonte 1900

*Anopheles argyrotarsis* albipes Theobald 1901

*Anopheles dubius* Blanchard 1905

"There seems to be no doubt as to the fact that *A. albimanus* is an important transmitter of malaria..." quoted from Army Medical Bulletin No. 59.

Field notes:

Adults: A large dark mosquito.

### 2. ANOPHELES (NYSSORHYNCHUS) AQUASALIS Curry 1932

Important synonyms:

*Anopheles tarsimaculata* Auct.

*Anopheles gorgasi* Dyar & Knab 1907

"*A. aquasalis*... is probably an important vector of malaria..." quoted from Army Medical Bulletin No. 59.

Field notes:

Adults: Resembles *oswaldoi* from which it can

only be distinguished by markings on 2nd hind tarsal segment.

### 3. ANOPHELES (NYSSORHYNCHUS) STRODEI Root 1926

Important synonyms: *Anopheles evansi* Brethes 1926

Field notes:

Adults: Adult female indistinguishable from *aquasalis* except by black white ratio on 2nd hind tarsal segment.

### 4. ANOPHELES (NYSSORHYNCHUS) TRIANNULATUS Neiva and Pinto 1922

Important synonyms:

*Anopheles bachmanni* Petrocchi 1925

*Anopheles davis* Paterson & Shannon 1927

*Anopheles perez* Shannon & Del Ponte Dyar 1928

Field notes:

Adults: A moderately large mosquito. Whitish scales on fore leg. Much like *albimanus*.

### 5. ANOPHELES (NYSSORHYNCHUS) ANOMALOPHYLLUS Komp 1936

Field notes:

Adults: Adult female can be distinguished from *aquasalis* only by black-white ratio on 2nd hind tarsal segment. Atlantic shores of Northern Panama. Quite similar to *aquasalis*.

### 6. ANOPHELES (NYSSORHYNCHUS) OSWALDOI Peryassu 1922

Important synonyms:

*Anopheles aquacaelestis* Curry 1930

*Anopheles tarsimaculata* Auct. (in part)

Field notes:

Adults: Resemble *aquasalis*, from which the adult female can only be distinguished by markings on 2nd hind tarsal segment.

### 7. ANOPHELES (NYSSORHYNCHUS) ALBITARSIS Lynch Arribalzaga 1878

Important synonyms:

*Cellia brasiliensis* Chagas 1907

*Cellia allopha* Peryassu 1921

Field notes:

Adults: A large light colored mosquito. Two parallel lines of white scales on anterior aspect 1st abdominal segment.

### 8. ANOPHELES (NYSSORHYNCHUS) ARGYROTARSIS Robineau-Devoidy 1827

Important synonyms: *Cellia rooti* Brethes 1926

Field notes:

Adults: Anterior aspect of 1st abdominal segment bare, dark colored; without lines of white scales.

### 9. ANOPHELES (STETHOMYIA) KOMPI Edwards 1930

Important synonyms: *Stethomyia nimbus* Auct.

Not Theobald

*Field notes:*

*Adults:* A slender black specimen. Black thorax with a narrow, white median line running from neck to scutellum. Body without scales. The tarsi of all the legs are black.

10. *ANOPHELES* (*ANOPHELES*) *PARAPUNCTIPENNIS* Martini 1932

Important synonyms: *Anopheles chiriquiensis* Komp 1936

This species is known to us by description only.  
*Field notes:*

*Adults:* The tarsi of all the legs are black. No scales on dorsal aspect of abdomen. In Panama, only found in Chiriqui.

11. *ANOPHELES* (*ANOPHELES*) *PSEUDOPUNCTIPENNIS* Theobald 1901

Important synonyms:

*Anopheles franciscanus* McCracken 1904

*Anopheles peruvianus* Tamayo 1907

*Anopheles argentinus* Brethes 1912

*Anopheles tucumanus* Lahille 1912

*Field notes:* A very large grayish long legged mosquito.

*Adults:* The tarsi of all the legs are black. No scales on dorsal aspect of abdomen. Thorax dark brown with a very broad grey central stripe from neck to scutellum: an important diagnostic point.

12. *ANOPHELES* (*ANOPHELES*) *EISENI* Coquillett 1902

Important synonyms:

*Anopheles tibiamaculata* Neiva 1906

*Anopheles niveopalpis* Ludlow 1920

*Field notes:*

*Adults:* A dark, fairly large mosquito. The tarsi of all the legs are black. No scales on dorsal aspect of abdomen. The white marking on the apical portion of the hind tibia is diagnostic. The costa of the wing is entirely black.

13. *ANOPHELES* (*ANOPHELES*) *VESTITIPENNIS* Dyar and Knab 1906

*Field notes:*

*Adults:* A large brown mosquito. This species is known to us by description only. There are no lateral abdominal sclae tufts.

14. *ANOPHELES* (*ANOPHELES*) *APICIMACULA* Dyar and Knab 1906

Important synonyms: *Anopheles maculipes* Dyar 1925 not Theobald

*Field notes:*

*Adults:* A moderately large, dark brown mosquito. All parts of the legs are speckled irregularly with yellow or white. The dark spot on wing apex is nearly diagnostic, locally. The wing shows the

costa with a prominent kink at the junction with the subcosta.

15. *ANOPHELES* (*ANOPHELES*) *PUNCTIMACULA* Dyar and Knab 1906

Important synonyms:

*Anopheles strigimacula* Dyar and Knab 1906

*Anopheles malefactor* Dyar and Knab 1907

"It is believed that *A. punctimacula* is an efficient vector of malaria at least in unsanitated regions in Panama" quoted from *Army Medical Bulletin* No. 59.

*Field notes:*

*Adults:* A large brownish mosquito. There are no scales on anterior aspect of 1st abdominal segment. All parts of all the legs are speckled irregularly with yellow or white. The wing shows the costa with a prominent kink at the junction with the subcosta.

16. *ANOPHELES* (*ANOPHELES*) *NEOMACULIPALPUS* Curry 1931

*Field notes:*

*Adults:* A large brown mosquito. All parts of all the legs are speckled irregularly with yellow or white. The wing shows the costa with a prominent kink at the junction with the subcosta.

*Larvae:* A diffuse white stripe down the dorsum of thorax and abdomen.

17. *ANOPHELES* (*KERTESZIA*) *NEIVAI* Howard, Dyar and Knab 1917

Important synonyms:

*Anopheles hylephilus* Howard, Dyar and Knab 1917

*Anopheles boliviensis* Zetek 1917 not Theobald  
*Anopheles lutzii* Auct.

*Field notes:*

*Adults:* Hind tarsus with white bands on the segments but with scutellum evenly rounded. Thorax grey, with four (4) black lines. Abdomen without scales.

18. *CHAGASIA* *BATHANUS* Dyar 1928

*Field notes:*

*Adults:* A shaggy brown, medium sized mosquito. The scutellum is slightly trilobed, making this a positive diagnostic point in Panama, as *bathanus* is the only local *Anopheles* so formed.

Charts 1 and 2 and Plates 1 and 2 permit a direct visual approach to identification.

It will be noted that two blanks of legs, palps and wings and of "Tables of Preferences" are provided in order that species, discovered in the future, may be sketched in or written up. Also under "Field Notes" of each species, blanks are provided for additional notes.

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PLATES 1-6.

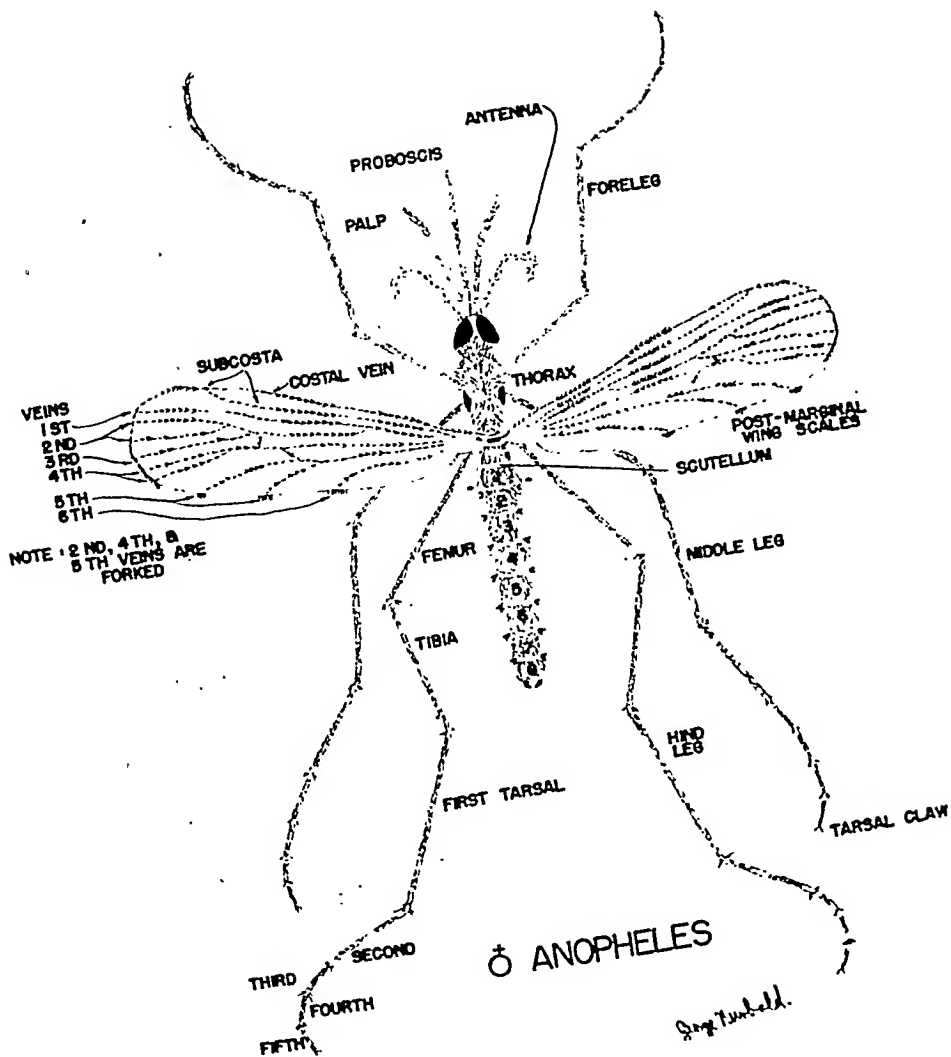




PLATE 2  
HIND LEGS (PANAMA SPECIES)

1. ALBIMANUS
2. AQUASALIS

3. strodei
4. triannulatus
5. anomalophylus

6. oswaldoi

*Series tarsimaculatus*  
In this series the 5th tarsal segment always has a basal black ring.

*Subgenus Nyssorhynchus*  
The 1st hind tarsal segment is all black; the 2nd basally more or less black, as shown; the 3rd and 4th all white and the 5th white, with or without a basal black ring.

7. albitarsis

*Series argyritarsis*  
In this series the 5th tarsal segment is always all white.

8. argyritarsis

9. kompi
10. parapunctipennis

11. PSEUDOPUNCTIPENNIS

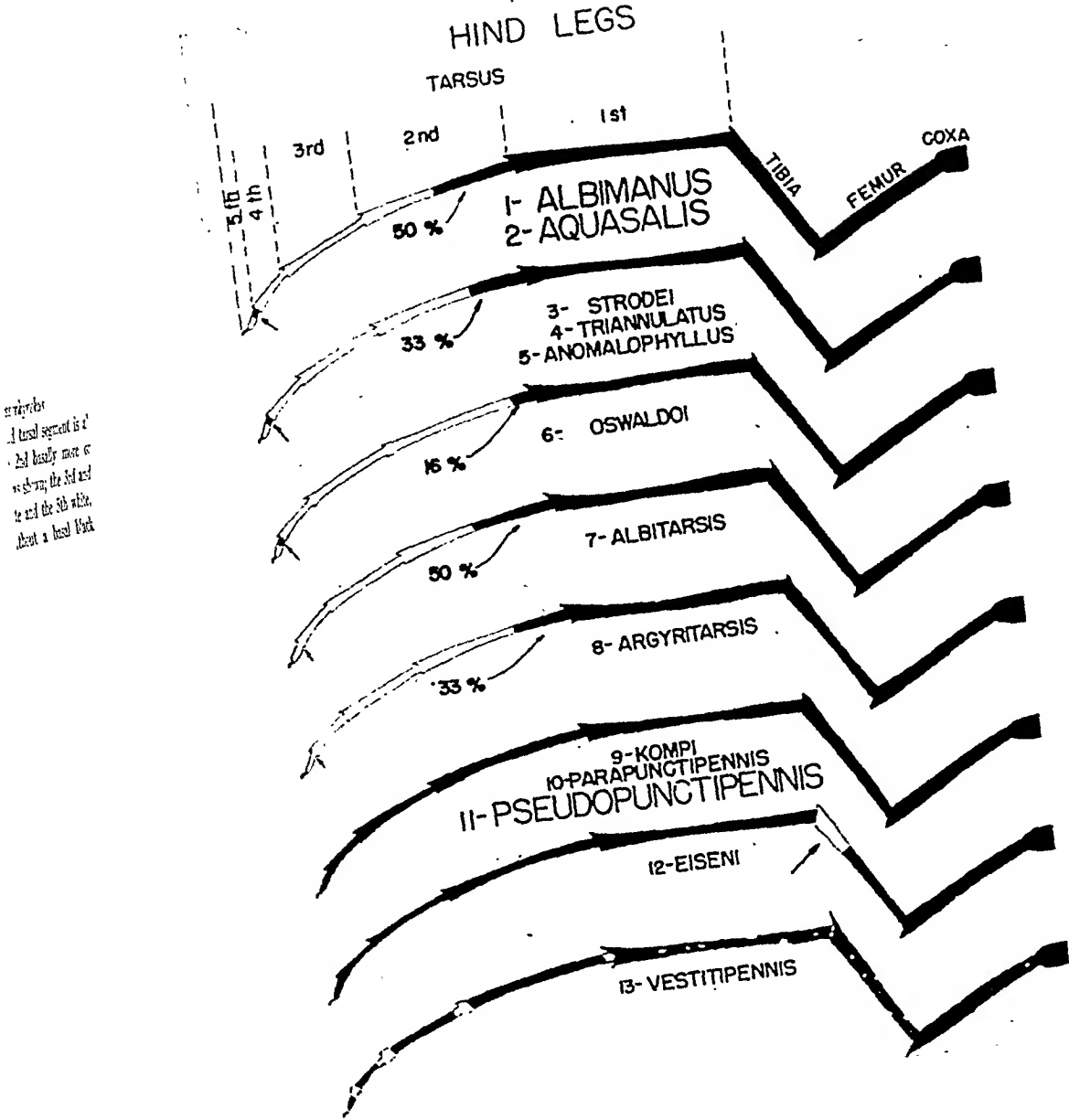
All five segments of the hind tarsus are all dark.

12. eiseni

13. vestitipennis

Narrow light rings at the tarsal joints.

THE ANOPHELES OF PANAMA  
C. P. BAXTER AND JAMES ZETEK



♀ PALPS

1- ALBIMANUS  
3- STRODEI  
7- ALBITARSIS  
8- ARGYRITARSIS

2- AQUASALIS  
4- TRIANNULATUS  
5- ANOMALOPHYLLUS  
6- OSWALDOI

12- EISENI

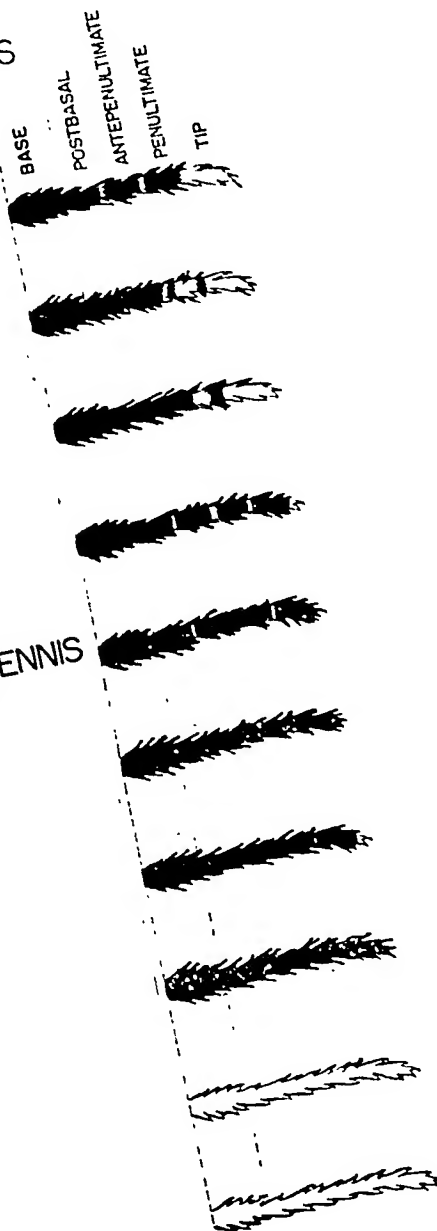
14- APICIMACULA  
16- NEOMAGULIPALPUS

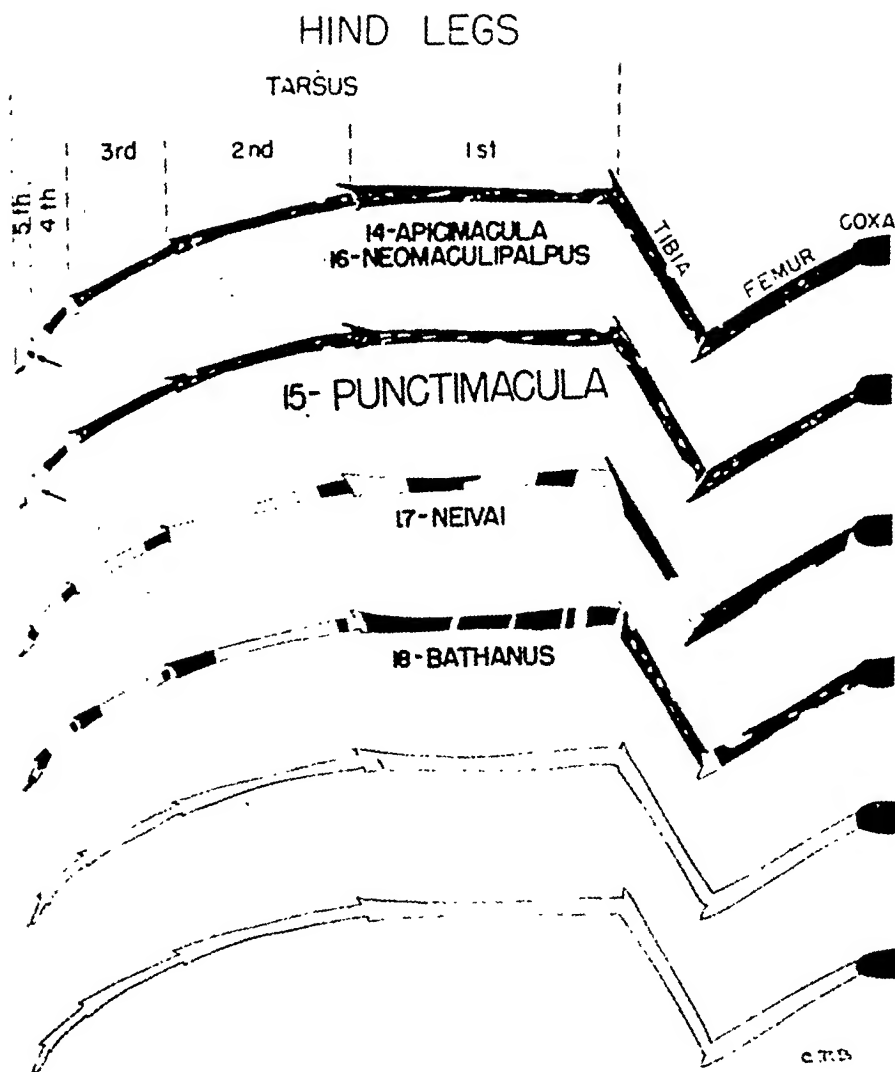
11- PSEUDOPUNCTIPENNIS  
15- PUNCTIMACULA

10- PARAPUNCTIPENNIS

17- NEIVAI

9- KONGI  
13- VESTITIPENNIS  
18- BATHANUS





WINGS



- 1- ALBIMANUS  
2- AQUASALIS  
3- STRODEI  
6- OSWALDOI  
5- ANOMALOPHYLLUS



- 4- TRIANNULATUS



- 7- ALBITARSIS  
8- ARGYRITARSIS



- 9- KOMPI



- 10- PARAPUNCTIPENNIS



- 11- PSEUDOPUNCTIPENNIS



- 12- EISENI



- 13- VESTITIPENNIS

WINGS



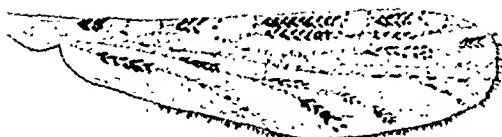
14-APICIMACULA



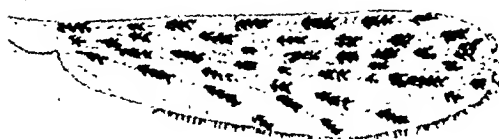
15-PUNCTIMACULA



16-NEOMACULIPALPUS



17- NEIVAI



18-BATHANUS





# THE ROLE OF THE RESERVOIR HOST IN TROPICAL DISEASE<sup>1</sup>

ELLIS HERNDON HUDSON<sup>2</sup>

*From the Tropical Disease Service, Naval Hospital, National Naval Medical Center, Bethesda 14, Md.*

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A reservoir host is a lower animal which shares some disease or parasite with man. The infection or the parasite is equally at home in man and the reservoir, is biologically indistinguishable in either case, and may oscillate quite contentedly from one to the other as environmental circumstances open the way. Such is *P. pestis* in relation to rat and man, and *E. granulosus* in relation to sheep and man. At certain times and places man may erect barriers of absolute defense and restrict the disease to the reservoir host, as with vaccination and mosquito control in yellow fever, or by abstention from uncooked fish as in clonorchiasis. He may go a step further and attack both the disease and the reservoir host itself, as in bovine tuberculosis and brucellosis in this country. However, if this effort fails of complete success, and if cracks appear in his personal walls of defense, the disease sweeps again into the human host.

In order to clear the ground and narrow this discussion to its proper limits five stipulations must be made at the start. First, we are not here concerned with intermediate hosts. In fasciolopiasis the snail and the plant are immaterial; the pig is the reservoir host. Second, susceptibility in an animal does not imply reservoir status. The experimentalist carries *T. pallidum* in rabbits and *E. histolytica* in kittens, but rabbits and kittens are not reservoir hosts of syphilis and amebiasis.

Third, many infections and infestations of man are so rare that they constitute medical curiosities. We may therefore disregard the dog harboring linguatula, the snake with porocephalus, the cat

with gnathostoma, the horse with rhinosporidium, the sheep with sarcocystis, and many others. In this group may be included "creeping eruption" and "swimmer's itch", due respectively to larvae of the dog hookworm and cercariae of avian schistosomes, whose activities are those of an aberrant parasite in an unsuitable host.

Fourth, the pig has an ascaris and a trichocephalus of his own and is not receptive to the analogous parasites of man, so he is not a reservoir in respect to these worms though they are respectively morphologically indistinguishable.

Finally, it is necessary to distinguish precisely between a vector and a reservoir host. It is true that in some cases, such as the ticks of relapsing fever and certain of the rickettsial diseases, the vector may also constitute a true reservoir. Usually, however, the short life-span of the vector, his inability to transmit the disease to his offspring, and his primary function of transport, suffice to exclude him from the reservoir status. For example, one may regard the phlebotomus, the louse, the tsetse fly, and the flea, as vectors but not reservoirs of leishmaniasis, typhus fever, African trypanosomiasis, and plague respectively.

Having now defined our subject both in positive and negative terms, we can proceed to a consideration of the animals which constitute disease reservoirs of man.

Hall once wrote that man acquires the diseases of lower animals when he "cuts across the lines of communication" utilized by the parasites in their movement from one to another customary host. There are three ways in which man may thus cut across nature's lines of communication. He may ingest food or water contaminated with parasites or their eggs on their way to lower animals; he may inadvertently expose himself to the bites of vectors similarly seeking their usual victims among lower animals; or he may acquire diseases from reservoir animals by contact or common environment.

From the standpoint of zoological classification, most parasites are comprised within four rather arbitrary groups, the helminths, the protozoa

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<sup>2</sup>Commander (MC), U. S. N. R.



(including the spirochetes), the bacteria (including the molds), and the viruses (including the rickettsias). These four groups are presented herewith in four tables which give the reservoir hosts of each parasite. Each group is subdivided in turn into three divisions on the basis of the parasite's

The reservoir hosts listed in these tables were gleaned from the standard textbooks on tropical medicine. The lists make no claim to be entirely comprehensive, and opinion in many respects is not unanimous, but details are immaterial to the present argument.

TABLE 1

*Helminths*

PARASITE		RESERVOIR HOSTS
a. Ingestion route		
<i>C. sinensis</i>	fish	Rat, mouse, mink, marten, badger, dog, swine, camel
<i>O. felinus</i>	fish	Dog, cat, swine
<i>H. heterophyes</i>	fish	Dog, cat, fox, wolf
<i>M. yokogawai</i>	fish	Dog, cat, swine, pelican
<i>P. westermanni</i>	crab	Dog, cat, fox, wolf, swine, panther, leopard, tiger
<i>F. buski</i>	plant	Dog, swine, rabbit
<i>F. hepatica</i>	plant	Sheep, cattle, buffalo, kangaroo
<i>G. hominis</i>	?	Swine, deer
<i>D. latum</i> (larva)	cyclops	Dog, cat, fox, wolf, swine, bear, walrus, seal, leopard, tiger
<i>T. solium</i> (larva)	flesh	Rat, swine
<i>E. granulosus</i> (larva)	eggs	Sheep, cattle, swine, camel
<i>H. nana</i>	eggs	Rat, mouse, gerbille
<i>H. diminuta</i>	eggs	Rat, mouse
<i>D. caninum</i>	eggs	Dog, cat, other carnivores
<i>D. medinensis</i>	cyclops	Fox, raccoon, mink in N. Am. Dog, wolf, skunk, horse, cattle, leopard, baboon in Old World
<i>T. spiralis</i>	flesh	Many rodents, ungulates and carnivores
b. Vector route		
	VECTOR	
<i>O. volvulus</i>	Simulium	Eland, antelope, deer, camel
<i>Loa loa</i>	Chrysops	Baboon
c. Contact		
	EXPOSURE TO	
<i>S. mansoni</i>	immersion (and oral)	Monkeys
<i>S. japonicum</i>	immersion (and oral)	Dog, cat, vole, swine, goat, sheep, horse, cattle

avenue of entrance into man, whether by ingestion, common vector, or common environment.

In a fifth table are listed the scientific and common names and the reservoir hosts of certain insects which, in adult or larval form, attack man when he intrudes between them and their normal hosts among the lower animals.

Reviewing the tables briefly, we observe that of the helminths which we share with reservoirs, we acquire most by the ingestion route, and very few by vector or contact. Most protozoans spill over from reservoirs to man by way of vectors, and hardly any by ingestion or contact. Bacterial parasites reach man by all three routes. Of those

virus and rickettsial diseases, however, which invade man from reservoirs, most employ vectors, a few are acquired by contact, and none at all through ingestion.

With characteristic anthropocentricity we regard the reservoirs as harboring "our" diseases, but actually the shoe is on the other foot. From the evolutionary standpoint most infections of man arose originally among the lower animals.

wonder grows that man has been able to achieve such a degree of isolationism as he has.

Let us now compare temperate and tropical zone medicine with respect to the role of the reservoir host. In civilized and temperate climates we still have the rabbit reservoir of tularemia, the rodent reservoir of sylvatic plague and spotted fever, the rat reservoir of endemic typhus and occasional leptospirosis, the dog reservoir of rabies,

TABLE 2  
*Protozoa (and spirochetes)*

PARASITE		RESERVOIR HOSTS
a. Ingestion route		
<i>B. coli</i> <i>L. icterohemorrhagiae</i>	cysts	Swine, rat, monkey, chimpanzee Rat, mouse, vole, bandicoot, dog, fox, leopard
b. Vector route		
	VECTOR	
<i>T. gambiense</i> <i>T. rhodesiense</i> <i>T. cruzi</i>	Glossina Glossina Triatoma, Rhodnius	Antelope, swine, goat, sheep, cattle Antelope, cattle, sheep, horse Armadillo, opossum, dog, cat, woodrat, bat, monkey
<i>L. donovani</i> <i>L. tropica</i> <i>L. braziliensis</i> <i>B. recurrentis</i>	Phlebotomus Phlebotomus Phlebotomus Ornithodoros (Spain and Africa)	Dog, hamster, cat, bear Dog Dog, agouti Rat, squirrel, other rodents, bat (Palestine)
<i>B. duttoni</i>	Ornithodoros	Old World: rat, weasel, shrew, hedgehog, squirrel, porcupine, dog, fox, jackal New World: Opossum, armadillo, monkey, marmoset, calf, horse, dog, fox
c. Contact		
	EXPOSURE TO	
<i>S. minus</i>	bite	Rat, mouse, weasel, squirrel, ferret, bandicoot, cat

The word reservoir therefore is appropriate in the time as well as the spatial sense. The animal kingdom is not only a storehouse of disease upon which we draw today, but it has been accumulating these diseases for countless ages. This reservoir is kept from flooding mankind by natural and man-made walls, but sometimes, because of internal pressures or because of breaks in the dam, they spill over into the domain of man's body. When one considers the multiplicity and ubiquity of parasitic forms in the animal kingdom the

the bovine reservoir of brucellosis, and the horse-and-fowl reservoir of equine encephalomyelitis. But none of these constitutes a grave menace to our national health. On the contrary, our chief struggles are with the diseases now indigenous to man, such as human tuberculosis, pneumonia, meningitis, diphtheria, syphilis, pyogenic infections, diabetes, cancer, and degenerative conditions such as arteriosclerosis, hypertension, and the psychoses.

The picture is different, however, when we turn

to the tropical regions of the earth and to the less highly organized countries of the temperate zone. Here are to be found of course the infections indigenous to man, but there is a marked reduction in the toll of degenerative disease and a striking increase in the number of diseases drawn from animal reservoirs. The animal reservoir host plays a prime role in tropical medicine.

answer to this question we are immediately struck by the lack of precise information in this field. It is true that perusal of any book on tropical diseases reveals the ever-recurring theme of the reservoir; but at few points is the theme developed. Instead, it is often confined to a mere list of animals; it is dismissed in a sentence hidden in the text in connection with other matters, or relegated

TABLE 3  
*Bacteria (and molds)*

PARASITE		RESERVOIR HOSTS
a. Ingestion route		
<i>M. tuberculosis</i> (bovine)	milk	Cattle
<i>B. melitensis</i>	milk	Goat, sheep
<i>B. abortus</i>	milk	Cattle
<i>Salmonella</i>	flesh	Swine, horse, fowls, rodents, carnivores, ruminants
<i>Streptococci</i>	milk	Cattle, goat, sheep
b. Vector route		
	VECTOR	
<i>P. pestis</i> (bubonic)	<i>X. cheopis</i> , <i>C. fasciatus</i> , etc.	Rat, vole, shrew, chipmunk, jerboa, gerbille, muskrat, tree and ground squirrel, cavy, bandicoot, tarbagan, marmot
<i>P. tularensis</i>	<i>D. venustus</i> , Chrysops	Grouse, ground birds, rat, mouse, rabbits, ground squirrels, other rodents; sheep, coyote, other mammals
<i>B. bacilliformis</i>	Phlebotomus	Dog
c. Contact		
	EXPOSURE TO	
<i>P. pestis</i> (pulmonary)	air	Hibernation—marmot. In epidemics—cattle, swine, sheep, donkey, deer, camel
<i>B. melitensis</i> , <i>abortus</i> , <i>suis</i>	contact	Horse, deer, buffalo, dog, goat, sheep, cattle, swine, fowls
<i>P. tularensis</i>	contact	Rodents and game
<i>B. anthracis</i>	air, contact	Dog, cattle, swine, sheep, horse, wolf, deer
<i>B. mallei</i>	air, contact	Horse, cattle, sheep, cat, dog
<i>Actinomyces</i>	contact	Cattle
<i>C. immittis</i>	air, contact	Wild rodents, cat, dog

It is important to emphasize this contrast in these days when large numbers of our men are living in those regions and when we may expect many to return with diseases acquired there. By way of preparation to meet this situation, we should be mobilizing our knowledge concerning the animal reservoirs out of which such infections spring. How complete is this knowledge?

If we approach tropical medicine in search of the

to fine print or a footnote. Why should the very source of man's diseases be dismissed in this cursory fashion instead of being displayed in bold type?

Several causes for this apparent neglect of an important subject suggest themselves. In the first place there is sheer lack of knowledge of the fundamental biology of the animal reservoir hosts; and where there is lack of knowledge there is lack of appreciation. Veterinary medicine, compara-

TABLE 4  
Viruses (and rickettsias)

PARASITE		RESERVOIR HOSTS
a. Ingestion route		
None		
b. Vector route		
	VECTOR	
Virus of Yellow fever	<i>Aedes aegypti</i> , Hemogogus, Sabethine, etc.	"Marsupials, edentates, rodents"; opossum, capybara, agouti, paca, sloth, anteater, arma- dillo, mouse, monkey Horse, birds, domestic fowl
Encephalomyelitis, equine and "St. Louis"	<i>Aedes</i> and <i>Culex</i>	
Rift Valley fever	<i>Tripanosoma</i>	Sheep, goat, cattle
Louping ill	<i>I. ricinus</i>	Sheep, mouse
Rickettsiae of		
Murine typhus	<i>X. cheopis</i>	Rat, squirrel, shrew
Spotted fever	<i>D. andersoni</i> , <i>D. variabilis</i>	Hare, mt. rat, chipmunk, ground squirrel, lynx, badger, coyote, goat, sheep, bear
Sao Paulo	<i>Amblyomma</i>	Opossum, rabbit, agouti, wild and domestic dog
Fièvre boutonneuse	Rhipicephalus	Dog
Q fever	? tick	Bandicoot
Tsutsug'ushi and scrub typh.	Larvae of <i>Trombicula</i>	Rat, mouse, hare, woodchuck, bandicoot, fowl, pheasant, dog, cat, buffalo
c. Contact		
	EXPOSURE TO	
Virus of Psittacosis	air, contact	Psittacines, canary, pigeon
Rabies	bite	Dog, cat, jackal, bat, meercat, genet, skunk, weasel, stoat, rabbit, fox, wolf, cattle, goat, swine, sheep, horse
Smallpox	? air, contact	Cattle

TABLE 5  
Insects

PARASITE	COMMON NAME	RESERVOIR HOSTS
<i>Auchmeromyia</i> (larva).....	Congo floor maggot	Many vertebrates
<i>Cordylobia</i> (larva).....	Tumbu fly	Rat, dog
<i>Chrysomya</i> (larva).....	Screw worm	Many vertebrates
<i>Dermatobia</i> (larva).....	Ver macaque	Dog, swine, cattle, monkeys, birds
<i>Oestridae</i> (larva).....	Bot flies	Horse, other equines
<i>Sarcophaga</i> (larva).....	Flesh fly	Many vertebrates
<i>Stomoxys</i> .....	Stable fly	Horse, cattle
<i>Tunga penetrans</i> .....	Sand flea	Birds and mammals, especially swine

tive pathology, and mammalogy exist, but these branches of science have entered but tentatively into the tropical field, and such knowledge as they

can contribute has not been integrated into the science of human disease. Obviously, facilities for research in these sciences have been scanty,

and restricted in time, geographical extent, and financial support. It is true of tropical medicine as a whole, and not only in respect to reservoir hosts, that impressions often pass for facts, and hypotheses for laws. The remedy is more research; ratiocination cannot take the place of adequate, relevant, and controlled data.

Another reason for the slight we have paid to the reservoir host is our preoccupation in tropical medicine with the symptoms of the sick patient. Following the conventional medical tradition we have concentrated first on diseases as they occur in *man*. Indeed, in many respects we have concerned ourselves with the diseases of the *white* man in the tropics. On the whole, tropical medicine is clinical or bedside medicine. Only here and there, when compelled by circumstances, does it approach a given disease as a whole, and relegate the sick individual to his proper subsidiary position.

This limited approach has not been peculiar to those who practice medicine in the tropics, but has been general. Medical science as a whole has been going through an evolution in its basic philosophy. The present generation of medical students is being diverted from the myopic and exclusive bedside view, and is being trained in the biological and sociological approach to disease, with emphasis on host-parasite-environment perspectives, and on prevention as well as cure.

Tropical medicine should be leading the development of medical thought in this direction. It deals with huge numbers of human victims encompassed

by an intricate web of animal and insect life, in a physical environment of extraordinary complexity and variability. Here if anywhere is a challenge to think of a disease as a biological entity embedded in a matrix of biological factors, and not a mere congeries of facts about symptoms, pathology, and diagnosis.

Related to the foregoing is the third reason for apparent disregard of the significance of the reservoir host. This is the relatively minor role permitted to epidemiology in tropical medicine. Objection to this statement may be made by those who will cite tropical diseases in which the epidemiology is known. Granted that there are such; but there is little reason for pride in the total picture. Any one can grasp the superficial epidemiology of many tropical diseases, and one cannot practice tropical medicine without being willy nilly some sort of epidemiologist. But Hall said of the helminths of man that, with the exception of seven worms, epidemiological knowledge is either "entirely lacking or highly inadequate", and it is incontrovertible that, on the whole, fundamental knowledge of what is called the "herd aspect" of most tropical diseases is deficient.

Tropical medicine furnishes a roster of great physicians and scientists, but it has yet to produce its great epidemiologist and environmentalist, and the definitive textbook on the epidemiology of tropical medicine has yet to be written. One of the principal chapters in that book will deal with the reservoir hosts of diseases affecting man.

# FEEDING HABITS OF THE PROVEN AND POSSIBLE MOSQUITO VECTORS OF WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS IN THE YAKIMA VALLEY, WASHINGTON<sup>1</sup>

W. C. REEVES AND W. McD. HAMMON

*From the George Williams Hooper Foundation, University of California, San Francisco*

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Repeated isolations of Western equine and St. Louis encephalitis viruses from mosquitoes caught in the Yakima Valley, Washington (1, 2, 3) have made it desirable to know more concerning the mosquitoes' feeding habits so as to give some idea as to the species of vertebrates serving as possible sources of virus. It was expected that infected mosquitoes would have fed frequently on those vertebrates which were serving as the reservoirs of these viruses. Neutralization tests on blood samples from the Yakima area during 1941 were interpreted as indicating which vertebrate species were the most frequently infected (4). Considerable evidence pointed to domestic fowl as the most important single group of animals (5, 6).

During the first entomological survey of the Yakima Valley, in 1941, *Culex tarsalis* Coquillett was found infected with Western equine and St. Louis viruses; and this species was demonstrated to best fit the epidemiological picture as a potential mosquito vector. Subsequent to this finding, the species was demonstrated to be an efficient laboratory vector of both viruses (5, 6, 7). A short series of blood smears from engorged *Culex tarsalis* were studied by the precipitin test method during the 1941 survey (8). The results indicated it had a wide host range which included the cow, horse, dog, chicken, man, pig and sheep. The first two species, the cow and the horse, were its most frequent hosts. The number of blood smears tested was small, 65, so evaluations of host preferences were not believed to be too significant.

A second survey of the mosquito vectors of

encephalitis was made in the Yakima Valley during 1942. Approximately fifty additional isolations of Western equine and St. Louis viruses were made from *Culex tarsalis* (3). In addition, *Culex pipiens* Linnaeus was found infected, once with St. Louis and once with Western equine virus; Western equine virus was isolated once each from *Culiseta inornata* (Williston)<sup>2</sup> and *Anopheles maculipennis freeborni* Aitken. Since *Aedes* have been suspected as vectors, a large sample was collected. None was demonstrated to have been infected. With these findings great importance was attached to comparative studies on the feeding habits of the various species of mosquitoes found in this area.

There were two possible methods of studying the vector feeding habits; hand collections of mosquitoes feeding on various vertebrates, and the precipitin test applied to wild-caught engorged females. The precipitin test appeared to offer the best possibility for the present studies. It has commonly been used in studies of the feeding habits of blood-sucking insects, and it has been a popular method of studying the feeding habits especially of Anopheline mosquitoes.

## METHODS

During the 1942 survey a series of 788 blood smears were made. This series represented 9 species of mosquitoes collected in domestic habitats and traps. Blood smears were tested with the sera of domestic rabbits immunized with the sera of the horse, cow, sheep, dog, man, and chicken. For greatest specificity in preparation of the antisera the method developed by Wolfe (9) was employed.

<sup>1</sup> This investigation was carried out in collaboration with the Commission on Neurotropic Virus Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Division, Office of the Surgeon General, U. S. Army; and under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development, and the University of California. Aided by a grant from the National Foundation for Infantile Paralysis.

<sup>2</sup> Formerly *Theobaldia*. According to Freeborn and Brookman in Identification Guide to the Mosquitoes of the Pacific Coast, U.S.P.H.S., Malaria Control in War Areas, May 1943 (10): "*Theobaldia* N-L. 1902 is invalidated by the prior use of *Theobaldia* Fischer 1887 . . . . As *Culicella* and *Culiseta* were proposed—by Felt in 1904, *Culiseta* takes priority because it was used by the first reviewer (Dyar 1921). . . ."

All of the antisera had a titre of 1:6400 or more. The antisera against horse, dog, and man were highly specific at dilutions above 1:100. Those against the cow and sheep were highly specific at dilutions above 1:800. Routine ring tests were carried out by layering a normal saline extract (1.0 cc. to each blood smear) of the blood smear antigen over a 1:5 dilution of each antiserum. Readings were taken at fifteen-minute intervals, and tests were considered positive when a precipitate developed at the interface within an hour. As expected some cross reactions occurred in the cases of the cow and sheep, which represented a

## RESULT

In table 1 the results of the precipitin test studies are presented. The figures on certain species *Aedes nigromaculis* (Ludlow), *Culiseta incidens* (Thomson), and *Anopheles punctipennis* (Say) are limited in their value because of small numbers. However, the samples from the other species, which are those we are primarily concerned with in this study, are more ample (over 50 smears each); and the findings are probably of some significance. *Culex tarsalis* and *Culex pipiens* were the two species most commonly found feeding on the domestic fowl (a reservoir of the encephalitic

TABLE 1  
*Precipitin tests on engorged mosquitoes  
of the Yakima Valley, 1942*

MOSQUITO SPECIES	BLOOD SMEARS REACTING TO SPECIFIC ANTISERA													
	Horse		Cow		Sheep		Dog		Man		Chick		No reaction	
	Per cent	Total number	Per cent	Total number	Per cent	Total number	Per cent	Total number	Per cent	Total number	Per cent	Total number	Per cent	Total number
<i>Culex tarsalis</i> ....	7.8	27	34.7	120	0.3	1	3.2	11	0.3	1	45.7	158	9.5	33
<i>Culex pipiens</i> ....	3.8	2	7.5	4	0.0	0	1.9	1	0.0	0	75.5	40	11.3	6
<i>Aedes vexans</i> ....	20.0	10	56.0	28	0.0	0	0.0	0	0.0	0	4.0	2	20.0	10
<i>Aedes dorsalis</i> ....	41.4	24	55.2	32	0.0	0	0.0	0	0.0	0	0.0	0	5.2	3
<i>Aedes nigromaculis</i> .....		0		4		0		0	0.0	0		0		5
<i>Culiseta inornata</i> ..	22.1	19	62.6	55	2.3	2	1.2	1	1.2	1	0.0	0	10.5	9
<i>Culiseta incidens</i> ..		1		4		0		1		0		0		0
<i>Anopheles maculipennis freeborni</i> .....	32.9	53	43.5	70	1.9	3	1.9	3	1.2	2	5.6	9	13.0	21
<i>Anopheles punctipennis</i> .....		7		10		0		0		0		0		2
Total .....														788

well recognized cross-species reaction. In such cases the ring reaction was always much stronger in one or the other of the antisera, and the first and stronger reaction was considered positive. The chicken antiserum gave ready response to other fowl bloods and could not be considered specific for chicken blood. However, a large number of those tests which gave positive reactions to chickens gave negative results when tested against an anti-duck serum prepared in chickens. This antiserum had a titre of 1:1600. Control tests were done on 9 known species of vertebrates.

Limited host range studies were made by hand collection of mosquitoes feeding on horse, cow and man.

viruses); however, both species included other common domestic animals and man in their feeding range. *Culex tarsalis* is sufficiently diverse in its feeding habits to include most of those domestic vertebrate species demonstrated by the neutralization test to have a high proportion of their members with immune reactions to St. Louis and Western equine encephalitis viruses (4). The feeding habits of this species alone might explain the distribution of encephalitis antibodies in this area. *Culex pipiens* was not nearly as common as *C. tarsalis* and in this area could only play a minor role in transmission of the encephalitides.

The results of the precipitin tests, along with the repeated isolation of virus from *Culex tarsalis*,

and antibody findings in chickens and other fowl, give strong support to the probability that domestic fowl are the important reservoir of infection in the Yakima Valley. Other species of mosquitoes: *Culiseta inornata*, *Anopheles maculipennis freeborni*, *Aedes dorsalis* (Meigen), *Aedes vexans* (Meigen) and *Aedes nigromaculis*, which feed mainly on large mammals, either were rarely found infected or were not demonstrated to be infected. This is probably an indication that large domestic mammals are not satisfactory sources of infective blood meals for the arthropod vectors. It may be that the virus never attains a sufficiently

Washington, a large series of blood engorged specimens, collected in domestic habitats, were tested by means of the precipitin method to determine the relative proportions which fed on domestic animal reservoirs of these infections and on man. At the same time, hand collections were made on the horse, cow, and man to determine which of the various mosquito species fed on these hosts.

It was found that *Culex tarsalis*, the species best fitting the epidemiological picture as a mosquito vector of encephalitis in the Yakima Valley, fed frequently on domestic fowl and included most of the common domestic animals and man in its

TABLE 2

*Mosquitoes collected from man and domestic animals, Yakima, Wash., 1941, 1942*

HORSE			COW			MAN		
Mosquito species	Per cent of total catch	Number caught	Mosquito species	Per cent of total catch	Number caught	Mosquito species	Per cent of total catch	Number caught
<i>Aedes vexans</i>	61.5	1322	<i>A. dorsalis</i>	48.7	366	<i>A. vexans</i>	73.3	179
<i>Aedes dorsalis</i>	14.3	307	<i>A. vexans</i>	31.0	233	<i>C. tarsalis</i>	14.8	36
<i>Culiseta inornata</i>	7.5	162	<i>A. maculipennis</i>	7.2	54	<i>C. inornata</i>	7.8	19
<i>Culex tarsalis</i>	5.9	126	<i>C. inornata</i>	6.1	46	<i>A. dorsalis</i>	3.3	8
<i>Anopheles maculipennis</i>	5.3	115	<i>C. tarsalis</i>	4.4	33	<i>C. incidens</i>	*	1
<i>Anopheles punctipennis</i>	3.5	76	<i>A. nigromaculis</i>	1.7	13	<i>A. maculipennis</i>	*	1
<i>Aedes cinereus</i>	0.8	18	<i>A. increpitus</i>	0.4	3			
<i>Aedes lateralis</i>	0.7	15	<i>A. cinereus</i>	*	1			
<i>Aedes nigromaculis</i>	0.1	4	<i>C. pipiens</i>	*	1			
<i>Culiseta incidens</i>	0.1	3	<i>C. incidens</i>	*	1			
<i>Culiseta morsitans</i>	*	1						
<i>Culex pipiens</i>	*	1						
<i>Mansonia perturbans</i>	*	1						
Total.....	100.00	2151		100.00	751		100.00	244

\* Species represented in collections by 1 specimen.

high titre in their blood to be infective to a mosquito. There is some experimental evidence to support this view.

Hand collections made on horse, cow, and man, table 2, demonstrated the large number of mosquito species feeding on these animals. It will be noted that *Culex tarsalis* was included in all these collections, however, as it was the most abundant species in the area and not the most commonly collected species from these animals, it would appear to be substantiated that neither the horse, cow, or man are its "preferred" hosts.

#### SUMMARY

As part of an epidemiological study of the mosquito vectors of encephalitis in the Yakima Valley,

feeding range. The feeding habits of this species alone could result in the incidence of encephalitis antibodies demonstrated in domestic animals and man in the Yakima Valley.

Species (including *Aedes*) which were rarely or never found infected in nature appeared to be those which fed almost exclusively on mammalian blood. *Culex pipiens* was the exception as it fed almost exclusively on fowl. This species, demonstrated to be capable of transmitting only the St. Louis virus, probably plays an important role for this one virus in areas where it occurs in large numbers.

The results of the precipitin tests, and the repeated isolations of virus from *Culex tarsalis* give strong support to the probability that domestic



fowl are an important reservoir of infection in the Yakima Valley.

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# ACUTE DYSENTERY PRODUCED BY SHIGELLA ALKALESCENS

## REPORT OF A CASE WITH NECROPSY

R. H. RIGDON,\* I. D. MICHELSON, AND FRANK ALLEN

*From the Departments of Pathology and Bacteriology, University of Tennessee, Memphis, Tennessee*

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There is some doubt that *S. alkalescens* is the etiological agent in any case of acute dysentery (1, 2). Felsen and Wolarsky (3), however, in 1940 correlated the clinical and bacteriological findings in 14 cases of diarrhea and concluded that "It seems advisable to regard *Shigella alkalescens* as a potential or actual pathogen." Welch and Mickel (4) have investigated two outbreaks of bacillary dysentery due to *S. alkalescens* among students and nurses. Brown and Anderson (5) cultured *S. alkalescens* from stools during three outbreaks of enteric disease and concluded that this bacterium may be associated with disease. The observations of several investigators (6-11) support the opinion that *S. alkalescens* may be pathogenic for man, although it has been cultured from the stools of apparently normal individuals (5, 8, 12). This organism is considered also to be the etiological agent in certain cases of pyelonephritis (6, 13, 14) and endometritis (15).

The bacteriological and immunological characteristics of *S. alkalescens* have been established unquestionably since it was first described by Andrewes (1) in 1918 (2, 4, 6, 12). Edward (16) in 1940 studied the effect of *S. alkalescens* on rabbits, guinea pigs, and mice and found that it required relatively large amounts of the culture to produce either symptoms or death. One rabbit had hemorrhagic necrosis of the appendix and the ileo-caecal region while another had petechiae into the appendix and into the collections of lymphoid tissue in the intestines. Focal necrosis also occurred in the center of the lymph follicles in the intestines. Necrosis likewise occurred in the regional lymph nodes and in the liver of one rabbit.

De Assis (7) reported one fatality in a group of five clinical cases of dysentery resulting from *S. alkalescens*. There were no pathological findings given, however, in his case. In this paper

we are reporting the pathological finding in a fatal case of acute dysentery in which *S. alkalescens* was isolated at autopsy from the colon.

### CLINICAL EXAMINATION

W. W., a colored male 14 months of age was brought to the hospital on the third day following a sudden attack of diarrhea. More than 10 stools were passed both day and night since the onset. They were small, watery, and contained a large amount of blood and mucus. During the three days preceeding hospitalization the baby had fever and vomited frequently. There were no convulsions.

On admission the temperature was 103.8°F., pulse 140 and the respirations were 26. The baby weighed 19 pounds. He was dehydrated. The reflexes were sluggish. There was some tenderness in both flanks.

The baby survived for only 20 hours following admission. The temperature remained elevated during the period of hospitalization. He gradually became comatose and the abdomen became distended and tympanitic. Immediately preceding death the abdomen was board-like in rigidity. During hospitalization the stools were small and frequent; they consisted primarily of blood and mucus.

### PATHOLOGICAL EXAMINATION

The autopsy was made four hours following death. Approximately 45.0 cubic centimeters of a clear straw colored fluid were present in the abdominal cavity. The intestines were moderately dilated. There were many focal areas of necrosis in the mucosa of the lower half of the ileum. These necrotic areas involved apparently every Peyer's patch and many of the solitary lymph follicles in ileum (figs. 1 and 2). The mucosa between the necrotic areas was normal. The mucosa between the ileocecal valve and the anus resembled that of the lower portion of the ileum. Essentially

\* Department of Pathology, School of Medicine, University of Arkansas, Little Rock, Arkansas.

every lymphoid follicle was hyperplastic and the mucosa covering these follicles usually was necrotic. These areas of necrosis in the colon varied from 1 to 3 mm. in diameter. The mucosa be-

The liver weighed 400.0 gms. The hepatic cells were swollen and small vacuoles were present in their cytoplasm. No areas of necroses were found in the liver. Both kidneys were similar,



FIG. 1. The ileocaecal region showing hyperplasia of the lymphoid tissue in the Peyer's patches and in the solitary follicles of the colon.

tween the hyperplastic follicles was normal (fig. 3). Clusters of gram negative bacilli were present in the necrotic tissue and sometimes they extended down into the submucosa. Mononuclear cells similar to large lymphocytes infiltrated the tissue at the base of these necrotic areas (fig. 4). Polymorphonuclear leucocytes in moderate numbers also were present. A few large mononuclear cells, the nuclei of which varied in shape from round to oval were present at the base of these necrotic areas. The nucleus of these large mononuclear cells stained lightly with hematoxylin. Many of the cells in the inflammatory exudate were pyknotic and fragmented. This cellular reaction was present in the submucosa and frequently it extended into the muscle in the ileum.

The lymph nodes in the mesentery were slightly enlarged and hemorrhagic. The sinuses in the cortex of these nodes were filled with innumerable gram negative bacilli while only a few bacteria were present in the medulla (fig. 5). The cellular reaction in the lymph nodes was insignificant. The nodes around the head of the pancreas were similar to those in the mesentery.

The spleen weighed 50.0 gms. It was dark red in color and moderately firm in consistency. The pulp did not bulge over the cut margin. The splenic sinuses were readily seen microscopically. Red blood cells and few splenic cells were present in the pulp. A few cells in the center of the Malpighian bodies were pyknotic and fragmented. No foci of necroses were found in the spleen.



FIG. 2. The margin of one of the Peyer's patches. Note the extensive necrosis with only a small amount of superficial desquamation. 18X.

together they weighed 150 gms. The epithelial cells lining the convoluted portion of the renal tubules were swollen. An albuminous precipitate was present in the lumen of many of the tubules. There was no inflammatory reaction in either the parenchymatous tissue or in the renal pelvis.

There were focal areas of atelectasis in both lungs. In the right there was a small calcified

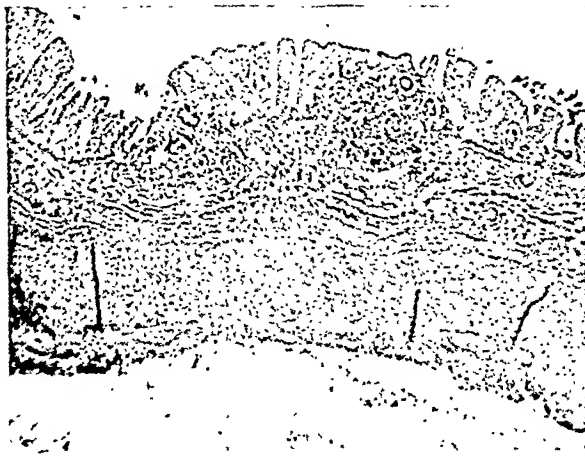


FIG. 3. Hyperplastic and necrotic follicles in the colon. There is no inflammatory reaction in the mucosa between these follicles. 18X.

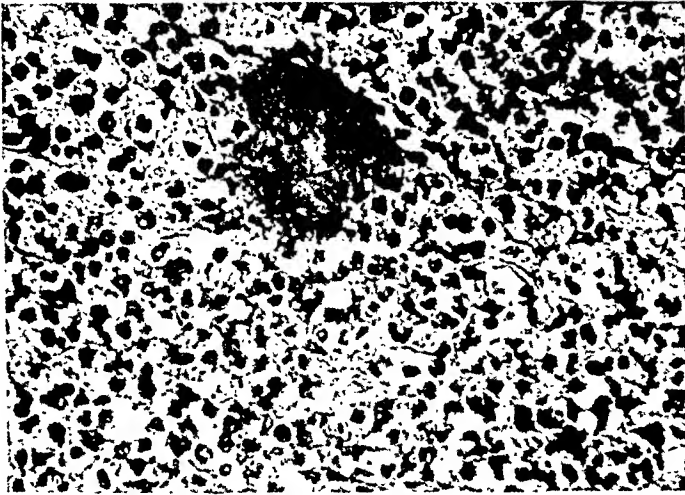


FIG. 4. Groups of gram negative bacilli are present in the intestinal lesions. Mononuclear cells and polymorphonuclear leucocytes are the principle cells in the reaction. This section is from the base of a Peyer's patch. 368X.

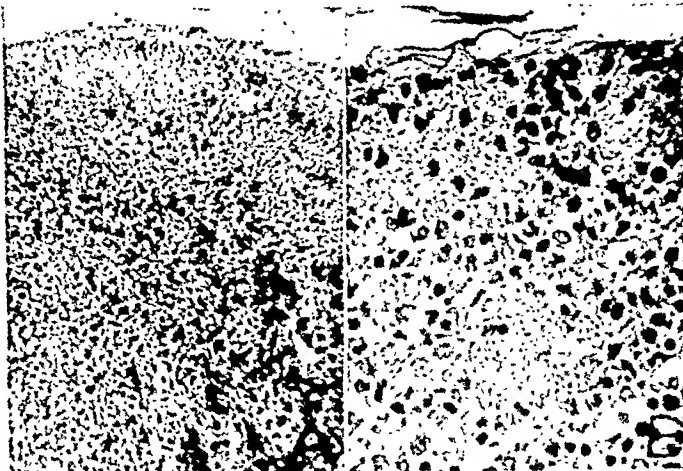


FIG. 5. A. The mesenteric lymph nodes are hemorrhagic and a zone of gram negative bacilli are present in the cortex immediately beneath the capsule. 72X.

B. Same as A. 368X.

area 0.5 cm. in diameter. Apparently it was an old tubercular lesion. The hilar lymph nodes were slightly enlarged and hemorrhagic. There were focal areas of edema in the lungs but no pneumonia.

There were no significant pathological changes observed in the remaining viscera.

Anatomical Diagnosis: Acute ileo-colitis; Etiology, *Shigella alkalescens*; Parenchymatous degeneration of the viscera.

Proskauer reaction was negative. The growth on plain broth was characterized by a diffuse turbidity without pellicle formation. Agglutination to a titer of 1:1280 was obtained in an anti-flexner serum, having a titer of 1:5120 against *S. flexner*.

The degree of alkalinity reached in litmus milk was less than that associated with *S. alkalescens*. The positive indol test, the fermentation of dulcitol, rhamnose and xylose excluded the possi-

TABLE 1  
*Agglutination titer of rabbit serum against S. alkalescens*

RABBIT NUMBER	EXPERIMENTAL DAYS									BLVD FOR SERUM	TITER
	1	5	6	7	13	15	20	21	26		
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	days	
4	1.0*		0.5		0.5			1.5	1.5	32	37°C. 1- 80 56°C. 1-2560
7	2.0	2.0		2.0		1.5	1.5			26	37°C. 1-2560 56°C. 1-2560+
9	3.0	2.0		2.0		1.5	1.5			26	37°C. 1- 320 56°C. 1-2560+
10	3.0	2.0		2.0		1.5	1.5			26	37°C. 1-1280 56°C. 1-2560+

\* The inoculum is an 18 hour broth culture.

Density of suspension No. 3 Standard McFarland, living organisms used.

Final dilutions carried out to 1:2560 only.

Controls saline suspensions carried out with each test.

Duplicate tests made on each series. One was incubated 37°C. (water bath) for 6 hours; ice-box overnight. The other series at 56°C. for 6 hours and ice-box overnight.

#### BACTERIOLOGICAL EXAMINATION

The organism isolated from the colon at autopsy was a gram negative rod, non-motile; non-spore bearing; and non-encapsulated. Morphologically, it resembled that of members of the enteric group. The reaction on Russell's Double Sugar was alkaline slant, and acid butt, without gas. The following sugars were fermented to the acid stage only: Dextrose, maltose, mannite, xylose, rhamnose and dulcitol. On litmus milk, an initial faint acidity was followed by a slow reversion to alkalinity. The degree of alkalinity reached, however, was not marked. Neither clot nor peptonization was noted. Indol was formed from tryptophane. Gelatin was not liquefied. No growth occurred in sodium citrate broth. The Methyl-Red test was positive and the Voges-

bility that this organism was *S. flexner*. The failure to ferment lactose, even after a 30 day incubation period, likewise excluded the other possibility, namely *Shigella madempensis*.

#### EXPERIMENTAL OBSERVATIONS

Rabbits, guinea pigs, and dogs were injected with broth cultures of *S. alkalescens* isolated from the colon of this infant. Ten rabbits were injected intravenously with 0.5 to 3.0 cc. of an eighteen hour culture at varying intervals during a period of one month. Four of these rabbits died and two were killed. Abscesses were found in the kidneys of some of these rabbits. No lesions were observed in either the intestines or the mesenteric lymph nodes in any of the animals. *S. alkalescens*

was cultured frequently from the blood and the contents of the colon.

The serum from four of these rabbits was used for agglutination against *S. alkalescens*. The titer, the quantity of the inoculum, and the dates are shown in table 1. The control titrations were always negative. The flocculi were much larger and more distinct when the sera were incubated at 56°C. rather than at 37.5°C. Each of the four rabbits as shown in table 1 gave a high titer.

Two dogs and four guinea pigs were given repeated injections of *S. alkalescens*. One of the guinea pigs died and one was killed. No significant pathological changes were found. The two dogs were killed. Several abscesses were present in the kidneys of one of these. No intestinal lesions were observed.

#### DISCUSSION

The pathological lesions in this case are different to any that we have observed in acute dysentery. It is difficult to believe that this patient was sick for only four days preceding death. The necroses in Peyer's patches resemble that observed in cases of typhoid fever dying between the 10th and 15th day of the disease. The diffuse involvement of the lymphoid follicles throughout the colon and too, the lymphoid tissue of the ileum, suggest a specific selectivity by *S. alkalescens* for lymphoid tissue. The mucosa between the follicles in the colon is normal in contrast to the diffuse involvement of the mucosa when Shiga and Flexner organisms are present.

The experimental lesions produced by Edward (16) in the rabbit are almost identical with those in this infant. Edward (16) did not consider that the results of his experiment contributed any evidence for believing *S. alkalescens* to be pathogenic for man. This opinion is also supported by the bacteriological studies obtained during several different epidemics of diarrhea in this country and abroad.

The clinical data are meager in this case; however, there does not appear to be any signs or symptoms to differentiate the dysentery produced by *S. alkalescens* from other types of acute dysentery.

It appears from a review of the literature that both the morbidity and mortality from *S. alkalescens* infection is low. Factors that may enhance the pathogenicity of *S. alkalescens* for man are not known at this time. Edward (16) suggested

that the lesions produced by *S. alkalescens* may result from an exotoxin similar to that produced by other organisms in the dysentery group.

#### SUMMARY

A case of acute dysentery in an infant 14 months of age is reported with the pathological findings. The etiological agent is considered to be *S. alkalescens*. The pathological changes are primarily in the lymphoid tissue in the colon and in the ileum.

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# MALARIA THICK FILMS CONTAMINATED WITH EXCRETIONS OF FLIES CONTAINING FLAGELLATES (*HERPETOMONAS*)

ARDZROONY A. PACKCHANIAN<sup>1</sup>

From U. S. Army LaGarde General Hospital, New Orleans, Louisiana

Received for publication May 3, 1943

Since the introduction of the thick blood film staining method for diagnosis of malaria, many surveys have been made in civilian and military groups for the detection of active and chronic cases of human malaria (1, 3, 4, 5).

In malaria surveys in which thick or thin film methods are used, one may occasionally be confronted with artifacts or other objects which may lead to an erroneous diagnosis. In the present communication the writer wishes to describe two cases in which blood smears were contaminated with *Herpetomonas*, apparently from fly excreta as confirmed by further laboratory studies.

## DATA

A blood film from a six-year-old negro in Alabama was sent to the writer for examination. This slide was well stained and contained a few flagellates, which in the opinion of some laboratory workers were considered to be *Trypanosoma cruzi*. However, in view of the absence of an undulating membrane and posteriorly located blepharoplast and parabasal body, these flagellates were diagnosed by the writer as *Herpetomonas* Sp., and because of the presence of bacteria on the stained smears, it was suspected that the smear was contaminated with excreta of some insect (Figure 1). To eliminate the possibility of this being a new disease of unknown etiology, the patient was examined by the writer and several additional thick and thin blood films were made. Also, about 20 cc. of blood were removed from the patient and inoculated into several N. N. tubes, mice and guinea pigs. All of the examinations gave negative results for flagellates.

Several hundred cage-bred house-flies (*Musca domestica*) were obtained from the U. S. Department of Agriculture. These flies were free from flagellate infection upon examination. The flies were placed into suitable glass cages. A suspen-

sion of a culture of *Herpetomonas muscae domesticae*<sup>2</sup> (2) from N. N. medium was placed in a well-glass slide and also on absorbent cotton and introduced into the cage with the flies. The flies readily ingested this material and all became heavily infested with *Herpetomonas*. After ascertaining that the flies were infected, twelve thick blood films were made and immediately placed in the cage containing the infected flies. They were observed feeding on the blood films and also defecating upon the slides and the smears. The slides were then stained according to the technique for thick films. Upon examination of the stained smears several specimens of *H. muscae domesticae* were encountered, whereas the slides not exposed to the infected flies were negative.

The second blood smear was obtained from a schoolboy from Mississippi during a malaria survey.<sup>3</sup> On examination the slide showed rather long herpetomonads and some bacteria was found which undoubtedly were deposited on the slide by some infected insect. The flagellates were stained beautifully, the nucleus was large and centrally located, and the blepharoplast was posterior to the nucleus. A long, well-stained flagellum was also observed but there was no evidence of an undulating membrane. The morphological characteristics of the flagellates were distinctly those of *Herpetomonas* or *Leptomonas*. There were no metacyclic trypanosome or crithidial forms. This slide was referred by the writer to Professor Frederick G. Novy of the University of Michigan, who confirmed the de-

<sup>2</sup>A culture of *Herpetomonas muscae domesticae* was received from Dr. R. Glaser of Princeton, New Jersey, during 1930. This strain has been maintained *in vitro* on N. N. media by the writer up to the present time (March, 1943). Subcultures have been made monthly or bimonthly.

<sup>3</sup>The writer's attention was called to this smear by Miss Aimee Wilcox of the National Institute of Health.

<sup>1</sup>Captain, Sanitary Corps, A. U. S.



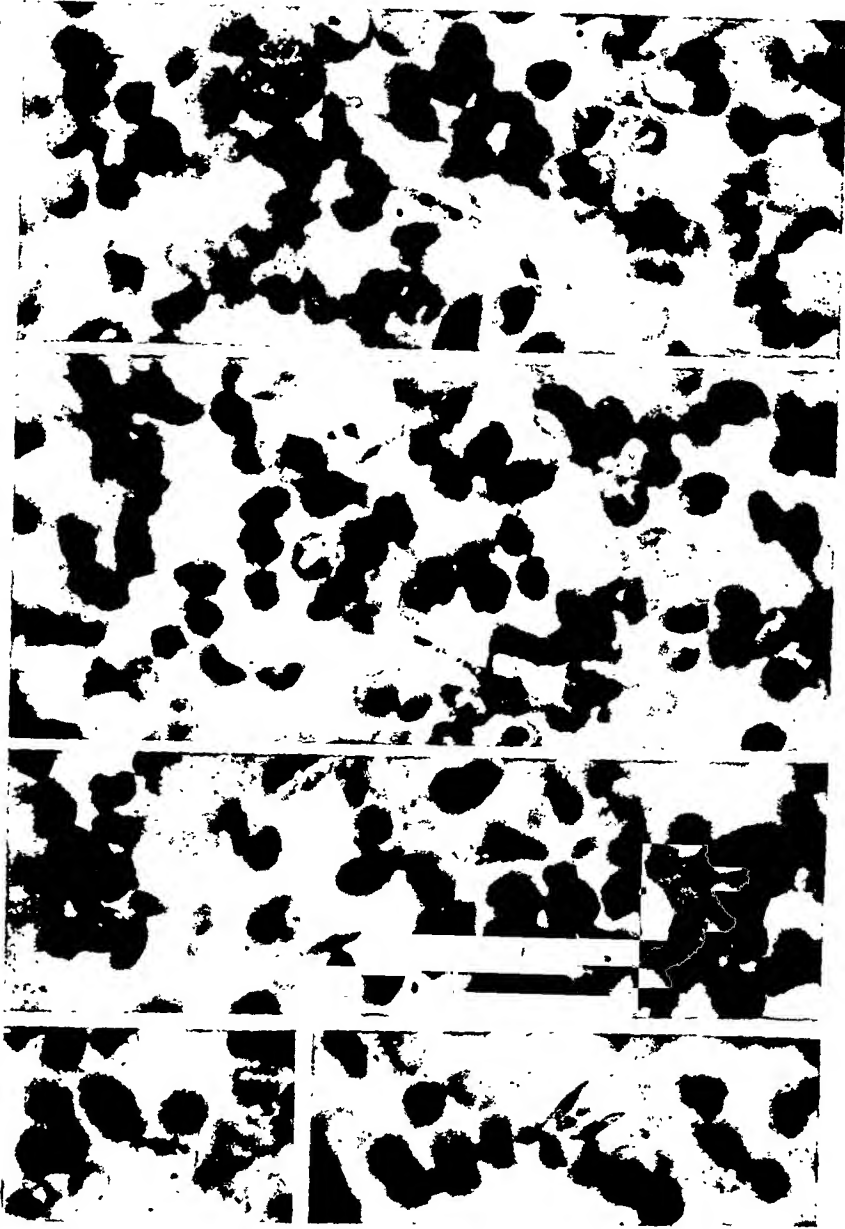


FIG. 1. Blood smear contaminated with *Herpetomonas* Sp.

cision that the smear was contaminated with the excreta of some insect.

#### DISCUSSION

In view of the fact that house-flies and other insects throughout the world may be infected with herpetomads or leptomonads and that they occasionally may contaminate malaria slides with flagellates and bacteria, it is obviously necessary that every precaution should be taken to protect the supply of the slides and the newly prepared

blood smears from flies and other insects. Such precaution not only avoids erroneous diagnosis but also prevents insects from eating the smears. In field work a slide box (capacity 25) held in an upright position will make an ideal container for storage of newly made thick films until the slides are dry (5).

When malaria smears are contaminated with *Herpetomonas* or *Leptomonas*, technicians may confuse them with (a) crescent of *Plasmodium falciparum*, particularly if the flagellum of the

parasite is not distinct; (b) with trypanosomas if not aware that in the blood of the vertebrate host trypanosomes do not occur in *Herpetomonas* or *Crithidial* forms.

#### SUMMARY

1. Two malaria thick films (one from Alabama and the other from Mississippi) were found contaminated with flagellates (*Herpetomonas* Sp.) which apparently were deposited on the smears by insects.

2. House-flies (*Musca domestica*) reared free of parasites were experimentally infected with a culture of *Herpetomonas muscae domesticae*. The experimentally infected flies were permitted to feed on freshly made thick blood film, which they contaminated with *Herpetomonas* by depositing their excretion on the smears.

3. In malaria surveys (particularly in the field), it is important to safeguard the newly made blood smears from house-flies and other insects in order

to avoid any possible confusion in diagnosis and in the destruction of the smears.

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## TRANSACTIONS

### THIRTY-NINTH ANNUAL MEETING

Meeting in Cincinnati, Ohio, in conjunction with the National Malaria Society and the American Academy of Tropical Medicine, as guests of the Southern Medical Association, November 16-18, 1943.

### BUSINESS MEETING

#### *The Minutes of the Council Meeting*

The annual business meeting (which was preceded by the annual dinner) of the officers and council of the Society was held at 6 p.m. on Monday, November 15, at the Gibson Hotel. Those

present were: Doctors C. F. Craig, J. S. D'Antoni, R. E. Dyer, N. P. Hudson, T. J. LeBlanc, A. C. Reed, W. A. Sawyer, J. S. Simmons, A. J. Warren. Those absent were: Doctors G. R. Callender, J. A. Curran, C. S. Stephenson, R. B. Watson. The president, Doctor N. P. Hudson, presided.

1. The minutes of the previous meeting, November 9, 1942, were accepted as published in the March 1943 issue of THE AMERICAN JOURNAL OF TROPICAL MEDICINE.

2. The president appointed Doctors Reed and LeBlanc as the Resolutions Committee.

3. The Secretary's report was presented as follows:

*Membership:* The total active membership of the Society as of November 1943, was 952, as compared with 650 on approximately the same date in 1942. The increment and other changes were as follows:

5 deceased (including 1 erroneously dropped as delinquent in 1942)

3 resigned (including 1 erroneously dropped as delinquent in 1942)

16 dropped as delinquent in 1943 (actually 21 were dropped, but the number is reduced by the 2 members erroneously classified as delinquent, but who actually were dead (1) or resigned (1), and by 3 others who reside in "mail service suspended" areas and who remain in status quo).

313 new members added by the Council in 1942 (actually the number was 314, but 1 member, through error, had re-applied for membership, and, because his initials and given names were stated differently, was re-accepted by the Council).

4 reinstated during 1943 (dropped as delinquent in 1942).

2 erroneously dropped as delinquent in 1942 and later found to be resident in "mail service suspended" areas.

7 accepted in 1942 but because of a change in the method of filing not appearing on the membership roster until 1943.

At the present time 38 members are 2 years delinquent and 102 are 1 year delinquent.

At present the Society has 4 emeritus and 20 honorary members. In addition (and in addition to subscriptions by libraries, institutions, etc.) 11 non-members subscribe to THE AMERICAN JOURNAL OF TROPICAL MEDICINE.

The roster of new members follows:

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BESS, GEORGE C.  
BEYER, EMIL CHARLES  
BIATTNER, RUSSELL JOHN  
BINGHAM, ELMER MCKINLEY  
BIRCH, CARROLL LAFLEUR  
BLACK, WILLIAM C.  
BLACKWELL, SAMUEL JOSEPH  
BLOUNT, ROBERT E.  
BLUMBERG, RALPH  
BORNSTEIN, JOHANN S.  
BOWEN, JOSEPH J.  
BRACKETT, STERLING  
BRAND, MAXWELL R.  
BRAUN, EMERY J.  
BRICENO-MAAZ, TULIO  
BRODKEY, MORRIS H.  
BROOKE, JAMES W.  
BROOKS, THOMAS JOSEPH JR.  
BROWNSON, BRADLEY C.  
BRUHL, CHARLES KENNEDY  
BUSTAMANTE, MIGUEL ENRIQUE  
CALERO, CARLOS MOLINA  
CARDENAS-MONTERO, MANUEL A.  
CARTER, FRANKLIN  
CASEY, CARLTON J.  
CAYAVES, PAUL G.  
CHERNOFF, HARRY A.  
CHYZANOWSKI, JOHN A.  
CLAPPER, MUIR  
CLAVIER, JORGE  
COCHRANE, CLELAND D.  
COCKERELL, EARL RUSH  
CONLIN, JOHN FRANCIS  
COOK, HUNTER SUMMERS  
CORRELL, WILLIAM C.  
COX, PAUL A.  
CROMARTIE, WILLIAM JAMES  
CUCKLER, ASHTON CLINTON  
CUSHING, EDWARD HARVEY  
DAVIS, JAMES ZIMMIRI  
DAVIS, PAUL VINCENT  
DAVISON, WILBURT CORNELL  
DELEON, DONALD  
DENTON, JAMES FRED  
DIGILIO, VICTOR A.  
DONOVAN, THOMAS JOSEPH  
DRAKE, WILLARD MELVIN JR.  
DULANEY, ANNA D.  
DWORIN, MORRIS  
DYER, EDWARD L.

- EDDY, HOWARD C.  
 EDELSON, EDMOND K.  
 EISENSTAEDT, WERNER F.  
 EPSTEIN, NORMAN  
 FALKINBURG, LE ROY WILKINS  
 FEDER, ISIDORE A.  
 FELSENFELD, OSCAR  
 FENTON, WARD C.  
 FERGUSON, MALCOLM S.  
 FINKELSTEIN, HERMAN  
 FINKELSTEIN, HYMAN M.  
 FINKELSTEIN, SAMUEL M.  
 FISHER, WILTON THOMROE  
 FLOOD, RANDOLPH GOVE  
 FORD, JOHN R.  
 FOURCHER, KENNETH R.  
 FOX, WILLIAM  
 FRAZIN, BERNARD  
 FREEMAN, GUSTAVE  
 FRICK, DAVID CLEMENTS  
 FURTH, JACOB  
 GABRIEL, FREDERICK RAPHAEL  
 GALT, JABEZ  
 GARBER, JARED YOUNG  
 GAUTHIER, AUGUST E.  
 GERMAN, WILLIAM MCKEE  
 GILLESPIE, JACOB E.  
 GOLDFINGER, MAURICE FREDERICK  
 GOLDSTEIN, DAVID H.  
 GOMEZ-MIRA, ADALBERTO  
 GONZALEZ-ALEJANDRO, LUJAN  
 GRACZYK, STEPHEN A.  
 GRAY, OTTO E.  
 GREENBERG, MILTON  
 GREENE, DAVID G.  
 GREENE, JAMES A.  
 GREENFIELD, IRVING  
 GREER, RICHARD HENRY  
 GROOT, HERNANDO  
 GUNDERSON, MILLARD F.  
 GUYTON, CLARENCE L.  
 HAGEMAN, HERBERT C.  
 HALEY, JOHN CARLIN  
 HAM, GEORGE HILLERY  
 HAMANN, CECIL B.  
 HAMMON, WILLIAM McDOWELL  
 HANDY, VINCENT HERBERT  
 HANNA, ROGER JULIEN  
 HARRELL, GEORGE T.  
 HARRELL, HENRY L.  
 HARRIS, ALBERT HALL  
 HAWKINS, PHILIP A.  
 HEAVENTRICH, ROBERT MAURICE  
 HEGARTY, FRANCIS A.  
 HERBERT, CHARLES T.  
 HERTZ, CARL  
 HETTING, ROBERT A.  
 HILL, RAYMOND M.  
 HOFFMAN, MORRIS JOSEPH  
 HOLDEN, EUGENE M.  
 HOLLADAY, BEVERLY L.  
 HOLMAN, RUSSELL L.  
 HOLMES, ROY H.  
 HOLZMAN, DANIEL  
 HOOK, WALTER E.  
 HORN, BENJAMIN  
 HORN, HENRY  
 HOSKINS, WILLIAM H.  
 HOWELL, JULIAN P.  
 HUDSON, ALFRED EDWARD  
 HUNTER, THOMAS H.  
 IHLE, CHARLES W.  
 INGALLS, MABEL S.  
 ISELIN, JOHN H. JR.  
 JACKSON, TRUXTON LAWRENCE  
 JAECHKE, WALTER HENRY  
 JAHNIG, RICHARD P.  
 JOHNSON, HERBERT BOLSTER  
 JOHNSTON, JOSEPH BOUDINOT JR.  
 JONES, GEORGE M.  
 JONES, JAMES B.  
 KAHN, JACOB PHILIP  
 KAMBHU, EKJAI  
 KAPLAN, LOUIS  
 KARCHER, JAMES FRANKLIN  
 KAUFMANN, WILLIAM  
 KEMP, ROBERT S.  
 KENNEDY, ROBERT A.  
 KERN, MAXIMILIAN  
 KHUON, ROBERT E.  
 KIEFER, NORVIN CHARLES  
 KING, JOHN H.  
 KLEINMAN, ABRAHAM I.  
 KLEINSCHMIDT, CLINTON CHARLES  
 KLINGER, SIEGFRIED  
 KNEEDLER, WILLIAM HARDING  
 KOHLENBERGER, CHARLES F. W.  
 KOSTMAYER, HIRAM W.  
 KYDD, DAVID M.  
 LAMBERT, LESLIE ANGELO  
 LAWRENCE, EDGAR A.  
 LEE, GEORGE R.  
 LEFEVER, KENNETH H.  
 LEHMAN, WILLIAM LOUIS  
 LEIGHTON, LESLIE H.  
 LEMERY, GEORGE W. JR.  
 LEVI-CASTILLO, JOSE ROBERTO  
 LEWE, IRVING A.  
 LICHTBLAU, ABE A.  
 LIEBERTHAL, MILTON M.  
 LILLICK, LOIS C.  
 LITTMANN, LEWIS E.  
 LONGNAKER, JOHN WILLIAM  
 LOPES, CID F.  
 LOWE, ROBERT C.  
 MCBREARTY, JOHN DENDY

McCARTY, WILLIAM C.  
 McDIVITT, MARCUS D.  
 McIVOR, BARBARA CATHERINE  
 MACDONALD, WILLIAM C.  
 MACKIE, JANET WELCH  
 MACKOWSKI, HERBERT WILLIAM  
 MANNING, ISAAC HALL JR.  
 MARCHEN-AQUIRRE, ALFONSO  
 MARTIN, JAMES R.  
 MELTON, JOSEPH  
 MERANZE, DAVID RAYMOND  
 MEYERS, GOLOMON G.  
 MIDENCE, ALFRED C.  
 MOLL, FREDERIC CLIFFORD  
 MOLLARI, MARIO  
 MONROY, BENJAMIN RON  
 MONTGOMERY, CHARLES CHRISTIAN  
 MOORE, DONALD V.  
 MORRISON, JOHN R.  
 MUELLER, JUSTUS FREDERICK  
 MUELLER, MORRIS A.  
 MULLOWNEY, JAMES PHILIP  
 NAPIER, LIONEL EVERARD  
 NOE, WILLIAM LEEDS  
 OCELUS, EDWARD V.  
 OLSEN, ALONZO Y.  
 OLSON, FREDERICK A.  
 OMSTEAD, MILTON H.  
 O'NEIL, JAMES T.  
 OYER, JOHN H.  
 PALMER, ALGER A.  
 PAPADAKIS, MARTHA GEORGIA  
 PAPINEAU, ALBAN  
 PARKER, JULIAN G.  
 PATTON, PAUL B.  
 PENNER, LAWRENCE RAYMOND  
 PERKIN, FRANK SCOTT  
 PIMENTEL-IMBERT, MANUEL FELIPE  
 PIXLEY, JOHN S.  
 PLATT, WILLIAM RADY  
 POMEROY, RICHARD W.  
 PRESS, EDWARD  
 RATHE, HERBERT WILLIAM  
 RAVELO DE LA FUENTE, JOSE JESUS  
 READ, HILTON S.  
 REESE, JOHN D.  
 REISSMAN, SEYMOUR  
 RILEY, FRANCIS S.  
 ROSENBERG, BENJAMIN  
 ROSENKRANTZ, JACOB ALVIN  
 ROSS, SAMUEL  
 RUBIN, IRA L.  
 RUEBUSH, TRENTON KIEFFER  
 RUSSELL, WILLIAM O.  
 SALADRIGAS, ENRIQUE  
 SARRO, NICHOLAS  
 SAWYER, LOGAN EVERETT  
 SCHERER, JOHN H.

SCHULER, WILLIAM HENRY  
 SCHUMANN, FRANCIS  
 SCHULZE, WILLIAM  
 SHANNON, PAUL V.  
 SHAW, ROBERT McLEOD  
 SHEINKOFF, JACOB ALLAN  
 SHEPARD, WAYNE  
 SHERSHOW, ALBERT  
 SHRAGER, JOEL  
 SHRAPNEL, BLISS CALCLIFFE  
 SILBERSTEIN, JACK S.  
 SIMPSON, MYRON L.  
 SKELLY, JOSEPH J.  
 SLAVIN, HOWARD B.  
 SMITH, CLARENCE WILLIAM  
 SMITH, EMERY VERNON  
 SMITH, FRANCIS D.  
 SMITH, MILTON S.  
 SPARHAWK, SAM  
 STADLER, HAROLD E.  
 STAFFIER, ANTHONY R.  
 STEIN, WILLIAM  
 STEINBERG, EDGAR IRWIN  
 STEWARD, WILLIAMS D.  
 STICH, MELVIN H.  
 STILWELL, GEORGE GILES  
 STONE, KNOWLTON D.  
 STOUT, HUGH A.  
 STRYKER, WALTER ALBERT  
 STUMP, ROBERT M.  
 SWANSEN, MERLE HARRIS  
 TAGER, BENJAMIN NATHAN  
 TAGER, MORRIS  
 TANNER, RONDELL H.  
 TAYLOR, EUGENE EMERSON  
 TEDROW, GEORGE C.  
 TUCKWILLER, PAT A.  
 VALENTINE, ELEANOR HARRIET  
 VAN LOO, JACOB  
 VEST, WALTER E. JR.  
 VICENS, CRISTOBAL A.  
 WALD, MILTON A.  
 WALL, EMMETT D.  
 WALLACE, WARREN S.  
 WAMPLER, FREDERICK JACOB  
 WASSELL, CORYDON McALMONT  
 WECHTEL, KARL N.  
 WEINBERG, JAMES IRVING  
 WEIR, JOHN M.  
 WEST, JOHN RICHARD  
 WHITE, MILLARD B.  
 WHITE, RAYMOND LEROY  
 WHITELEY, HORACE W.  
 WIESEL, BENJAMIN  
 WIKOFF, JOHN LESLIE  
 WILLIAMS, THOMAS HENRY  
 WILLIAMSON, GEORGE RALPH  
 WILLIS, WILLARD H.

WING, WILSON M.  
 WOLLENMAN, OSCAR JOHN JR.  
 WOOD, EDWARD NEIL  
 WOODS, ARCHIE SCOTT  
 WYBORNEY, EUGENE H.  
 YEATTS, HARRY BLAIR  
 YOOD, ALFRED  
 ZIMMERMAN, LOUIS

Those who resigned from active membership during 1943 are:

J. L. CARR  
 O. G. HAZEL  
 G. H. MILLER

With regret the names of the following members were listed as having died during the past year:

M. R. DINKELSPIEL  
 J. B. HELM  
 W. A. HOFFMAN  
 A. D. SELLARDS  
 H. ZEILER

Letters of condolence are sent to the families of deceased members whenever the Secretary's office is notified of the death.

#### *Action of the Council by Correspondence:*

(a) Approval by correspondence of the names of 314 applicants for membership in the Society during 1943.

(b) Approval of the publication by the Society of a bi-monthly bulletin, the dates of issue to alternate with the dates of issue of THE JOURNAL.

(c) Approval of traveling expenses to the meeting for the Editor of THE JOURNAL, Colonel Charles F. Craig.

(d) Approval of appointment by the President of a Committee on War and Post-War Activities.

*Circular Letters:* On December 18, 1942, 21 delinquent members were informed by registered mail of the action of the Council November 6, 1942. (Am. J. Trop. Med., 23:300, March, 1943). On January 31, 1943, letters to the general membership, in which bills for 1943 were included, were sent out by the Secretary. These letters dealt with the high-lights of the 1942 meeting and with other matters of general interest. On February 24 letters were sent to the Officers, Councilors and Committeemen by the President, requesting suggestions as to plans for the coming year as well as for the 1943 meeting; the replies were very helpful, and most of the suggestions have been included in the program. June 2 letters were sent by the Treasurer to delinquent members. July 26 letters

were sent by the President asking essayists to participate in the program. November 2 letters were sent to former Presidents of the Society inviting their attendance at the coming meeting.

*Appointment of Committees:* June 11, 1943, a Committee to elect a new Editor was appointed by the President, consisting of Doctors M. F. Boyd Chairman, and C. F. Craig, R. E. Dyer, A. C. Reed and W. A. Sawyer, for the purpose of selecting a member of the Society to replace Colonel Charles F. Craig as Editor of THE JOURNAL.

The Secretary expressed his sincere appreciation for the interest and cooperation shown by both the Council and the members during 1943, and especially thanked the President for his invaluable assistance during the entire year. Attention was again called to the excellent cooperation shown by the Southern Medical Association through the Secretary-Manager, Mr. C. P. Loranz. The Secretary also thanked committee chairman as well as committeemen for their assistance during the year. He called to the attention of the members the fact that the tremendous increase in growth of the Society had been largely due to the efforts of Colonel G. C. Callender and Lieutenant Colonel T. T. Mackie of the Army Medical School.

4. The following action was taken on the Secretary's report:

(a) 21 members delinquent in dues were dropped from the rolls.

(b) Resignations submitted by 3 members were accepted.

(c) The deaths of 5 members were noted with deep regret.

(d) The sum of \$125 was voted for secretarial assistance during 1942-43.

(e) The report of the committee to elect a new editor was tabled until the Editor's report had been read.

5. The report of the Treasurer was read (as follows) and accepted.

#### CHECKING ACCOUNT

##### RECEIPTS

Balance on hand, November 7, 1942.	\$490.66
Received from Eli Lilly & Co. for	
Bailey K. Ashford Award .....	1150.00
Transfer from Savings Account, State National Bank of Sheffield, Alabama to checking account of National Bank of Commerce.....	\$1268.96



Interest from Savings Account State, National Bank of Sheffield, Alabama..... 9.62

Total..... 1278.58

Less Checking Account in State National Bank of Sheffield transferred to Savings Account..... 306.61 971.97

Dues from membership to 1943..... 4347.25  
Dues from membership for 1944..... 70.00

Total..... \$7029.88

#### DISBURSEMENTS

To Editor, Charles F. Craig, for secretarial service for 1942-43 ... \$100.00

To Secretary, E. Harold Hinman for secretarial service from November 1, 1941 to April 3, 1942... 20.00

To Secretary, J. S. D'Antoni for secretarial service from April 3, 1942 to November 7, 1942 ..... 50.00

Walter Reed Medal Awards..... 23.55

Transportation expenses of Editor to 1943 meeting..... 92.13

To Norman H. Topping, expenses Cincinnati meeting (Bailey K. Ashford Award)..... 150.00

To Norman H. Topping, for Bailey K. Ashford Award..... 1000.00

Postage..... 108.00

Printing and Stationery..... 96.24

Refund to Members..... 49.88

Checks (not sufficient funds) ..... 10.00

Bank Charges..... 26.47

U. S. War Savings Bonds Series F:  
3 @ \$370.00 each .... 1110.00  
3 @ 74.00 each .... 222.00 1332.00

Check for collection..... 5.00

Williams & Wilkins, Publishers, for the Journal..... 3310.59

Incidental office expenses ..... 20.00

Total Disbursements..... \$6393.86

Balance on hand, November 7, 1943. 636.02

#### SAVINGS ACCOUNT

Balance on hand, November 7, 1942 ..... \$962.45

Interest..... 9.62

Transfer from checking account..... 306.61

Total ..... 1278.58

Balance transferred to checking account in National Bank of Commerce..... \$1278.58

#### ASSETS OF SOCIETY

Balance in checking account..... \$636.02

U. S. War Savings Bonds Series F..... 1332.00

\$1968.02

The Treasurer requested that he be authorized, with the President, to purchase war bonds with surpluses which might develop during the year after necessary expenses had been met. This request was granted.

Doctors Warren and Sawyer were designated to audit the Treasurer's books and to approve them if correct.

6. The report of the Editor of THE JOURNAL (Colonel Charles F. Craig) was presented as follows:

With the November issue, THE AMERICAN JOURNAL OF TROPICAL MEDICINE, the official organ of the American Society of Tropical Medicine, will have completed its twenty-third volume. The past year has been the most prosperous in the history of THE JOURNAL, because of the large number of new members who have been added to the Society, especially from the ranks of the Army, Navy and Public Health Service, and because of the realization by the medical profession of this country that a knowledge of tropical diseases will be of greater and greater value and importance, especially during the post-war period, when diseases peculiar to, or most prevalent in tropical regions will be brought home by returning troops who have served in these regions. This addition to the membership has, of course, resulted in a similar addition to the subscription list, which has practically doubled during the past few months. The character of the papers submitted for publication in THE JOURNAL has been high, and many excellent papers have had to be returned to their authors only because of lack of space for publication. The restrictions placed by the War Production Board upon the amount of paper used by publishers has greatly hampered THE JOURNAL and although efforts have been, and are still being, made to secure an increased allowance, the prospects for such an increase are anything but favorable at present.

During the past year a new contract was made with the publishers, in accordance with the action

of the Society at the last meeting, whereby the Society will share in any profits made by THE JOURNAL. It will be remembered that for many years the publishers continued to print THE JOURNAL at a loss, and at one time our debt to them amounted to several thousand dollars. This debt has been gradually reduced during the past decade and the Editor is pleased to be able to report that at the present time THE JOURNAL is entirely free from debt and that in 1943 the Society will receive a substantial profit in accordance with the provisions of the new contract.

Colonel Craig expressed his own sincere appreciation and the appreciation of the Society to the Williams & Wilkins Company which for so many years printed THE JOURNAL at a loss and thus prevented cessation of publication.

One Supplement to THE JOURNAL was published during the year. The policy of publication of papers paid for by the author, or by the institution with which he is connected, has been continued, and several of the most valuable papers appearing in THE JOURNAL during 1943 were thus published. The Editor expressed his appreciation to the authors of these papers and to institutions with which they are connected, especially the Rockefeller Foundation, and recommended that this policy be continued. It does not interfere with the publication of other papers, as all paid papers are printed as extra pages, and it does result in the publication of very valuable contributions that we would otherwise be unable to publish.

There has never been a time in the history of the country when a journal devoted to tropical medicine is as useful and essential as at the present time and the Editor thought that the Society could be congratulated upon publishing a journal that is the only such journal published in this country and is recognized as one of the best in this field published in any country. He thought the future very bright for THE JOURNAL owing to the increased importance of tropical medicine to the medical profession in the United States.

In tendering his resignation as Editor of THE JOURNAL Colonel Craig desired to express his appreciation of the encouragement and assistance given him through the 17 years that the Society had so honored him by the officers, members of the council, past and present, and by the members of the Society, and he wished for the new Editor the same cordial assistance that had been given him.

7. The following action was taken on this report:

(a) It was received with a vote of sincere ap-

preciation for the valuable work of the Editor in the past.

(b) Although the Officers and Council, as well as the membership, fully realized the hardships inherent in the position, Colonel Craig was asked to reconsider his resignation and resume his editorship of THE JOURNAL, which, after some discussion he agreed to do.

(c) It was decided that the Editor and the President of the Society should make a concerted effort to have the War Production Board reconsider its ruling on limitation of paper for THE JOURNAL, which otherwise would be greatly curtailed in size in 1944, because of the large increase in the membership of the Society.

(d) The sum of \$150 was voted for secretarial assistance.

8. The following committee reports were presented:

(a) The Committee on Honorary Membership recommended no election of new members at this time. This report was accepted.

(b) The report of the Membership Committee has been included in the report of the Secretary. No additional other members were elected at this time.

(c) The selection of Colonel George R. Callender to present the Eighth Charles F. Craig Lecture was approved and the Secretary was directed to extend the thanks and appreciation of the Society to the speaker.

(d) The report of the Program Committee was accepted.

(e) The Secretary was directed to inform the Committee on the Award of the Walter Reed Medal that this award will be available for the 1944 meeting.

(f) The presentation of the Bailey K. Ashford Award to Dr. Norman H. Topping of the National Institute of Health, as recommended by the Committee on Award, was approved.

(g) The report of the Committee on Teaching of Tropical Medicine was accepted as read. The suggestion of the Committee, that Dr. H. E. Meleney be selected as the new chairman, because of his outstanding contributions to under-graduate and post-graduate training in tropical medicine as chairman of the similar committee in the Association of American Medical Colleges, was also accepted after it had been voted to continue this committee.

(h) The report of the newly formed Committee on War and Post-War Activities was held in

abeyance, since formal plans could be made only after the symposium and special business meeting to be devoted to this subject.

(i) Since no meetings were held in 1943, no reports could be made by the delegates to the meetings of the councils of the American Society of Parasitology or the American Association for the Advancement of Science. If meetings should be held in 1944, Doctor Meleney was selected to represent the Society in the former and Doctors Faust and Clark in the latter Society.

(j) It was recommended to the Society by the resolutions committee that letters of appreciation be sent to the following individuals and organizations: Mr. C. P. Loranz, Southern Medical Association; Doctor Louis B. Owens, Hamilton County Medical Society; Doctor Harvey F. Garrison, Southern Medical Association; Mr. Randall Davis, Hotel Gibson; Colonel George R. Callender; and Colonel Charles F. Craig.

9. It was voted to continue affiliations with the Southern Medical Association and the National Malaria Society.

10. The council appointed Colonel Charles F. Craig to replace Colonel F. F. Russell and to serve for a period of 6 years on the Walter Reed Medal Committee.

11. Appointments made by the President for the Charles F. Craig Lecture Committee, Honorary Membership Committee, Program Committee and the Membership Committee appear on page 145, as do the appointments made by the council to the committee on the Teaching of Tropical Medicine.

12. The Council voted to propose the following ballot of new officers to the Society: President-Elect, R. E. Dyer; Vice-President, H. W. Brown; Council for 4 years, J. F. Kessel, O. R. McCoy; Council for 1 year, L. T. Coggeshall, to fill the unexpired term of Doctor Dyer who became President-Elect.

13. J. S. Simmons was selected as a member of the editorial board of *THE JOURNAL* for 5 years to replace J. Andrews, whose term expired.

14. There was no unfinished business to be brought before the council.

#### *New Business*

15. Doctor E. C. Faust was appointed to serve as the representative of the Society at business meetings of the American Foundation of Tropical Medicine, the appointment being made at the request of the Foundation.

16. Since the terms of the Bailey K. Ashford Award had never been published, it was voted that they be published in *THE JOURNAL*.

17. Adjournment followed.

#### *SPECIAL BUSINESS SESSION*

*November 17, 1943, 9:30 p.m.*

The Society, in conjunction with the American Academy of Tropical Medicine and representatives of the American Foundation of Tropical Medicine, met in special session to consider the responsibilities and opportunities of service in tropical medicine in the present war emergency and in the immediate future. Consideration of the subject was introduced by the report of the Committee on War and Post-War Activities of the Society in Tropical Medicine and the discussion was led by Dr. Coggeshall, Chairman of the Committee.

After a discussion of the many questions involved, each organization (with Doctor Hudson presiding for the American Society of Tropical Medicine) unanimously approved the following resolutions; namely,

1. That the committee on War and Post-War Activities of the Society be continued, to consider problems concerned and recommend appropriate action to the Society;

2. That the Society publish a so-called bulletin, bi-monthly and alternating with *THE JOURNAL*, to serve as a medium for distributing information related to the Society, tropical medicine, and the war emergency;

3. That the Society formulate and forward to the American Academy a resolution that graduate and under-graduate education and research in tropical medicine be energetically promoted now and in the future, and that this matter and its various ramifications be referred to the American Foundation for consideration and action, with the assurance that the Society wishes to assist as it is able, through its organization and individual members;

4. That the Society formulate and forward to the appropriate Army medical agencies resolutions expressing the Society's commendation and appreciation of the successful service of assembling and distributing parasitological and pathological specimens to institutions and individuals for use in education in tropical medicine during the present emergency, with the assurance that the Society, both through its organization and individual members, wishes to be of service as it can for the promo-

tion and continuation of these very successful services; and

5. That the Society send an expression of its sincere thanks and appreciation to the John and Mary R. Markle Foundation for its financial support of the teaching program in tropical medicine, without which this essential contribution to the emergency medical situation in the nation's military forces could not have been made.

#### ANNUAL BUSINESS MEETING

November 18, 1943, 12:15 p.m.

1. The minutes of the 1942 business meeting were accepted as published.

2. Transactions of the Council as set forth in items 3, 4, 5, 6, 7 and 12 were approved.

3. The actions of the second business session were also approved.

4. It was unanimously passed that the Secretary of the Society, by virtue of his office, should serve as the editor of the proposed new bulletin of the Society.

5. Adjournment followed.

#### SCIENTIFIC SESSIONS

The first scientific session of the Society was called to order at 2 p.m., Tuesday, November 16, in Parlor H of the Gibson Hotel by President, N. Paul Hudson, Columbus, Ohio. The program follows:

1. "Influence of Vitamin Intake upon Phagocytic Activity" by C. A. Mills and Ester Cottingham, University of Cincinnati College of Medicine, Cincinnati. Presented by Doctor Mills.

2. "The Role of the Reservoir Host in Tropical Diseases" Ellis Herndon Hudson, Commander, Medical Corps, USNR, U. S. Naval Medical School, Bethesda. Discussed by Doctors Rees and Packchianian.

3. "Epidemiology of Tropical Diseases in Mexico" Miguel E. Bustamante, Instituto de Salubridad y Endermedades Tropicales, Mexico.

4. The Eighth Charles F. Craig Lecture on Tropical Medicine: "Diarrheal Diseases", George C. Callender, Colonel, Medical Corps, USA, Army Medical School, Washington.

5. "The Reaction to Intradermal Trichinella Antigen in Patients with Tuberculosis", George T. Harrell, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem. Discussed by Doctor Bozicevich.

6. "Intradermal and Serological Tests with

*Dirofilaris immitis* Antigen in Cases of Human Filariasis", John Bozicevich, National Institute of Health, U. S. Public Health Service, and A. M. Hutter, Lieutenant Commander, Medical Corps, USN, U. S. Naval Hospital, Bethesda. Presented by Doctor Bozicevich and discussed by Doctor Huntington.

7. "The Treatment of Canine Heartworm (*Dirofilaris immitis*) with Anthiomaline", H. W. Brown, T. J. Brooks, Jr., and E. Waletzky, University of North Carolina, School of Medicine, Chapel Hill. Presented by Doctor Brown and discussed by Doctors Reed, Meleney, Bozicevich, Napier, Packchianian, Culbertson.

8. "The Diagnosis of Filariasis by Immunological Procedures, with Antigen from *Litomosoides carinii* of the Cotton Rat", J. T. Culbertson and H. M. Rose, Columbia University College of Physicians and Surgeons, New York. Presented by Doctor Culbertson and discussed by Doctor Bozicevich.

9. "Intradermal Reactions Following the Use of *Dirofilaris immitis* Antigen in Persons Infected with *Onchocerca volvulus*", Willard H. Wright and John R. Murdock, Pan American Sanitary Bureau, Washington. Presented by Doctor Wright.

The second scientific session of the Society was called to order at 9 a.m. in the ball room of the Gibson Hotel, November 17, 1943, by President N. Paul Hudson. The program follows:

10. "The American Foundation for Tropical Medicine", Alfred R. Crawford, Secretary, American Foundation for Tropical Medicine, Brooklyn.

11. "The Teaching of Tropical Medicine to Instructors in Medical Schools, A Report", Henry E. Meleney, New York University College of Medicine, New York.

12. "The Distributing Center for Parasitological Specimens", George W. Hunter, III, Major, Sanitary Corps, Army Medical School, Washington. Discussed by Doctors Sawyer and Ackert.

13. "West Coast Problems in War-Time Tropical Medicine", A. C. Reed, University of California Medical School, San Francisco. Discussed by Doctors Mustard, Faust, Bustamante, Kessel, Kessel, Kellersberger and Shattuck.

14. "Filarial Lymphagitis Among Troops in a South Pacific Archipelago", Robert W. Huntington, Jr., Lieutenant Commander (MC) USNR, R. H. Fogel, Lieutenant Commander (MC) USNR, S. Eichold, Lieutenant (MC) USNR, and James G. Dickson, Captain (MC) USN, U. S. Naval Hospital, San Diego. Presented by Doctor Hunt-

ington and discussed by Doctors Napier, Sandground, Brown and Coggeshall.

15. "Entomological Phases of Recent Dengue Outbreak in Honolulu", Robert L. Usinger, Passed Assistant Sanitarian (R), U. S. Public Health Service, Carter Memorial Laboratory, Savannah. Discussed by Doctors Napier, Hackett, Meleney, Salisbury, Bishop, Komp and Simmons.

16. "Yellow Fever Control During the War", Charles L. Williams, U. S. Public Health Service, New Orleans.

17. "The Introduction of Tropical Diseases into the United States After the War", W. A. Sawyer, Director, International Health Division, Rockefeller Foundation, New York.

18. "Current and Future Considerations of the Tropical Disease Field", L. T. Coggeshall, University of Michigan Medical School, Ann Arbor.

The third session was called to order Thursday, November 18, at 9 a.m. in the Club Room of the Gibson Hotel, by President N. Paul Hudson. President-Elect W. A. Sawyer presided. The program follows:

19. "Malaria in High Altitudes", Henry Hanson, Jacksonville. Discussed by Doctors Coggeshall, Hackett, and Bustamante.

20. "The Age Level for the Peak of Acquired Immunity to Malaria as Reflected by Labor Forces", Herbert C. Clark, Gorgas Memorial Laboratory, Panama. Discussed by Doctors Hackett and Napier.

21. "Amebiasis of the Uterus", Damaso de Rivas, Philadelphia. Discussed by Doctor Meleney.

22. "The Influence of Cholesterol and Certain Vitamins on the Growth of *Endamoeba histolytica* with a Single Specimen of Bacteria", Charles W. Rees, John Bozicevich, Lucy V. Reardon and Floyd S. Daft, National Institute of Health, U. S. Public Health Service, Bethesda. Presented by Doctor Rees and discussed by Doctors Topping, Bozicevich and Meleney.

23. "Comparison of Chlorine and Ozone as Cysticidal Agents of *Endamoeba histolytica*", John F. Kessel and Donald K. Allison, University of Southern California School of Medicine, Los Angeles. Presented by Doctor Kessel and discussed by Doctors Sandground and Rees.

24. Presentation by Doctor H. E. Meleney of the Bailey K. Ashford Award in Tropical Medicine to Norman H. Topping, Passed Assistant Surgeon, U. S. Public Health Service, National Institute of Health, Bethesda.

25. "Infantile Toxaplastic Encephalomyelitis: A Clinical and Pathological Study of Five Cases", William O. Russell, William P. Callahan and Margaret G. Smith, Washington University School of Medicine, St. Louis. Presented by Doctor Russell and discussed by Doctors Clark and Sakin.

26. "The Contamination of Natural Waters by *Pasteurella Tularensia*", R. R. Parker, Edward A. Steinhaus and Glen M. Kohls, Rocky Mountain Laboratory, U. S. Public Health Service, Hamilton. Presented by Doctor Steinhaus and discussed by Doctors Foshay, Faust and Packchianian.

27. "The Protean Manifestations of Weil's Disease", Muir Clapper and Gordon B. Myers, Wayne University College of Medicine, Detroit. Presented by Doctor Clapper and discussed by Doctors Meleney and Napier.

The following papers (numbers 28-31) were listed by title or (numbers 33-34) were not presented because of the essayists' inability to be present at the meeting:

28. "The Behavior of *Trichomonas vaginalis* in a Semi-Solid Medium", Mary J. Hogue, University of Pennsylvania School of Medicine, Philadelphia.

29. "A Report on a Case of Valantidiasis with Observations on Experimental Transmission to Rats and Mice", H. Tsuchiya and Bruce Kenamore, Washington University School of Medicine, St. Louis.

30. "A Fly-Borne Epidemic of Bacillary Dysentery in Bivouac Areas of an Army Camp", A. Packchianian, Captain, USA, Rhodes General Hospital, Utica.

31. "Blindness, Frequent in Lepers, is Always Due to Neglect", W. H. Hoffman, Finlay Institute, Havana.

32. "Leptospirosis in New Orleans", Harry Senekjic, Tulane University School of Medicine, New Orleans.

33. "Trypanosomiasis in Liberis", Everett P. Veatch, Pasadena.

34. "Protective Value of Intradermal Inoculation of Spotted Fever Virus and Homologous Immune Serum", Ludwick Anigstein, Madero N. Bader, Dorothea Naubauer and Gerald Young, University of Texas Medical Branch, Galveston.

#### JOINT SESSION WITH THE NATIONAL MALARIA SOCIETY

November 18, 1943, 2:00 p.m.

General J. S. Simmons, President of the National Malaria Society and Doctor N. Paul Hudson,

President of the American Society of Tropical Medicine, presided at this meeting. The program follows:

*Symposium on National Program for the Control of Malaria*

35. Address of President of National Malaria Society: "American Mobilization to Combat War-Time Hazards of Malaria", James S. Simmons, Brigadier General, MC, USA, Director, Division Preventive Medicine, Office of the Surgeon General, Washington.

36. "The Malaria Control Program of the Army", O. R. McCoy, Major, MC, USA, Office of the Surgeon General, Washington.

37. "The Malaria Control Program of the Navy", Omar J. Brown, Commander, (MC) USN. In Charge Section on Tropical Medicine, Division of Preventive Medicine, Bureau of Medicine and Surgery, Navy Department, Washington.

38. "The Malaria Control Program of the U. S. Public Health Service among Civilians in Extra-Military Areas", (Mr.) Stanley B. Freeborn, Senior Surgeon (R), U. S. Public Health Service, Malaria Control in War Areas, Atlanta.

39. "Malaria Control Activities of the Pan American Sanitary Bureau", Hugh S. Cumming, Director, Pan American Sanitary Bureau, Washington. Discussed by Doctor Moll.

40. "Malaria Control Activities of the Institute of Inter-American Affairs", G. C. Dunham, Brigadier General, MC, USA, Director, Division of Health and Sanitation, Institute of Inter-American Affairs, Washington.

41. "Facilities for the Training of Malariologists in Military and Civil Institutions", Henry E. Meleney, Professor of Preventive Medicine, New York University College of Medicine, New York.

42. "The Contributions of the Bureau of Entomology and Plant Quarantine to the National Program for the Control of Malaria", (Mr.) F. C. Bishopp, Assistant Chief, Bureau of Entomology and Plant Quarantine, Washington.

43. George R. Carden, Jr., Division of Medical Sciences, National Research Council, Washington, D. C. (title of paper not at present available).

44. "A Proposed Plan to Prevent the Spread of Malaria in the United States from Infected Individuals Returned from Abroad", W. A. Saw-

yer, Director, International Health Division, Rockefeller Foundation, New York.

45. "A Program for Eradication of Malaria in the United States", J. W. Mountin, Assistant Surgeon General, States Relation Division, U. S. Public Health Service, Washington.

OTHER EVENTS

1. The annual luncheon of the Society was held Wednesday, November 17, 1943, at 12:30 p.m. The President of the Society, Doctor N. Paul Hudson, Ohio State University, Columbus, Ohio, who was introduced by the President-Elect, Doctor W. A. Sawyer, presented as his presidential address: "A Broader Perspective for Bacteriology".

At the conclusion of his address he read the names of the former presidents and introduced those attending in commemoration of the Fortieth Anniversary of the Society. In addition, a silver vase was presented to Colonel Charles F. Craig by the Society for his untiring efforts in all phases of the work of the Society as well as Editor of THE JOURNAL.

Doctor E. C. Faust, Tulane University School of Medicine, New Orleans, presented a paper entitled "The American Society of Tropical Medicine: A Biographical Sketch of the First Forty Years".

2. Well attended hospitality group sessions were held on November 16 and 17 at 4:30 p.m. At the first session, Lieutenant Colonel Thomas T. Mackie spoke on reciprocal inter-American relationships, and at the second Doctor E. Harold Hinman presented the work of the Department of Inter-American Affairs in Latin America, with special reference to El Salvador.

3. The American Academy of Tropical Medicine held its tenth annual dinner at 7:00 p.m., Wednesday, November 17, to which all members of the Society were invited. Lieutenant Colonel Thomas T. Mackie of Washington, D. C., was toastmaster.

Doctor Lewis M. Hackett, Buenos Aires, Argentina, presented as his presidential address "The South American Scene".

Doctor Herbert C. Clark presented the Theobald Smith gold medal of the George Washington University School of Medicine to Colonel Charles F. Craig, San Antonio, Texas.



# THE AMERICAN JOURNAL OF TROPICAL MEDICINE

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*Editor, CHAS. F. CRAIG*  
Colonel, U. S. Army, Retired  
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## AWARD OF THE RICHARD PEARSON STRONG MEDAL FOR OUTSTANDING ACHIEVEMENT IN THE FIELD OF TROPICAL MEDICINE

The American Foundation for Tropical Medicine, Inc., announces the establishment of an award for outstanding achievement in the field of tropical medicine to be awarded periodically as circumstances determine. This award is to be known as the Richard Pearson Strong Medal and will consist of a paladium medal together with a cash honorarium of five hundred dollars, the gift of the Winthrop Chemical Company to the Foundation.

The first award was made at the annual meeting of the Foundation, on February 28th, 1944, to Colonel Richard Pearson Strong, Medical Corps, Army of the United States, for whom the medal was named, by Rear Admiral E. R. Stitt, M.C., U. S. Navy, Retired, former Surgeon General of the United States Navy. It was most appropriate that the first award of this medal should have been made to Colonel Strong, in recognition of his pioneer work in tropical medicine and for whom the medal was named. The citation for the first award of the medal was as follows:

The medal and award for distinguished achievement in tropical medicine has been established to honor outstanding contributors to this important field of the medical sciences. It is fitting that it should bear the profile and the name of a distinguished American physician who has devoted his career to this branch of medicine and whose name is known throughout the world. It is peculiarly appropriate that the first award should be made to him.

A scientist, who since his appointment in 1899 as President of the first United States Army Board for the Investigation of Tropical Diseases in the Philippine Islands, and subsequently as Director of the Philippine Government Biological Laboratory in Manila, has made

fundamental contributions to scientific knowledge of many tropical diseases, including bacillary and amebic dysentery; cholera; bubonic and pneumonic plague; beri beri; yaws; tropical ulcer and tropical skin diseases; trypanosomiasis; typhus fever; filariasis; onchocerciasis—the blinding filarial disease of Africa and Central America; and Oroya fever.

Author of many important scientific articles and monographs dealing with tropical diseases and of the revised edition of the most distinguished American text on tropical medicine.

Leader of scientific expeditions to remote areas of the tropics of Africa and of the Amazon Valley, to Central America and the valleys of the Andes.

Samaritan, physician and leader of relief expeditions to the peoples of Manchuria stricken by a devastating epidemic of pneumonic plague, and later to Serbia which was in the throes of the great epidemic of typhus fever in 1915.

Teacher and Professor of Tropical Medicine at the University of the Philippines from 1907 to 1913; Professor of Tropical Medicine at Harvard University from 1913 to 1938; and organizer of the first graduate School of Tropical Medicine in the Western Hemisphere.

Past President of the American Society of Tropical Medicine, American Academy of Tropical Medicine, the American Society of Parasitologists and the Association of American Physicians.

Eminent figure in military medicine; member of the Inter-Allied Sanitary Commission in the first World War; Consultant in Tropical Medicine to the Secretary of War; Director of the Course in Tropical Medicine at the Army Medical School; and member of the Medical Corps of the United States Army in four wars; recipient of the Distinguished Service Medal in 1919 for exceptionally meritorious and distinguished services, notably as President of the Board for the Investigation of Trench Fever—COLONEL RICHARD PEARSON STRONG.



# THE AGE LEVEL FOR THE PEAK OF ACQUIRED IMMUNITY TO MALARIA AS REFLECTED BY LABOR FORCES<sup>1</sup>

HERBERT C. CLARK

*From the Gorgas Memorial Laboratory, Panama, R. de P.*

Received for publication December 9, 1943

Malariologists are inclined to some range of opinion as to when the peak of immunity is gained by people who spend their lives in regions where malaria has high endemicity. Perhaps this is partly due to the fact that it is always more convenient and time saving to survey the children who are more apt to be present at home or in the schools. For many years I have personally been interested in the *labor index* of malaria and I think enough evidence has been assembled in some regions where I have had service to present it for analysis. It might be well to refer to a few of the records from well recognized authors from 1922 to 1942 before presenting this experience.

(1) *Byam and Archibald (1922)*. Immunity to malaria, when it is present, is almost always a partial immunity acquired by frequent infection and reinfection repeated continuously over a number of years. Children who survive to the age of three or four years will be found to have acquired a considerable degree of *tolerance* but parasites will continue to be found in their blood.

(2) *Manson-Bahr (1929)*. It has been shown that the natives of malarious districts acquire their immunity from repeated and persistent infection in childhood. In such places the blood of practically every child up to 3 or 4 years of age contains malaria parasites. The proportion of infected children gradually becomes smaller with each additional year until adolescence is approached, when the blood becomes practically parasite-free and immunity is established.

(3) *Boyd, Mark F. (1930)*. Absolute immunity in malaria appears to be rare. There is abundant evidence to indicate that a relative immunity may be acquired. The resistance shown by natives or old residents of a highly endemic area was first shown by Koch to be the result of infection, and associated with it. The acute infestation which

lasts through childhood to adolescence might be called the stage of *immune infestation*.

(4) *Hackell, L. W. (1937)*. Quotes Barber as having found 100 per cent of the children infected after their first year of life in Lagos and that young adults in their twenties complained from time to time of illness while those over 30 years showed little evidence of malaria.

(5) Djapardze made a study of immunity in malaria based on mass observation conducted in the course of two years (1927-8) in the Black Sea Coast of Caucasus where the three main species of parasites occur. In one district (Gal) no anti-malarial measures were in effect and the disease was hyperendemic. Its course could be observed in the true form. This district was compared with the Gudaut district where malaria was characterized by epidemic outbreaks with a low general incidence. He found that the population of the hyperendemic region, having been repeatedly exposed to seasonal infections in the course of 4 to 5 months from year to year, had developed a relative immunity which was strictly strain-specific. The parasite rate reached its maximum at the age of 5, after which it gradually decreased until by the age of 40 it was halved. When both benign and malignant tertian malaria were present, the population acquired a more stable immunity against the former. The immune state of the adult population in the hyperendemic region was reflected (1) in the absence of severe clinical forms of malaria and (2) in the presence of infected persons showing no symptoms at all.

In 1922 I was advised to use the following age groups in malaria surveys of labor camps and rural communities: 0-4 years, home or domestic life; 5-14 years, school period; 15-49 years, active business period of life; 50 years and over, inactive life.

It was believed that serious malaria control would only be necessary in the first two groups since acquired immunity would protect the others.

<sup>1</sup> Read at the Thirty-ninth Annual Meeting of the American Society of Tropical Medicine, Cincinnati, Ohio, November 16-18, 1943.

This made it appear that those of *labor age* would be easy to control in labor camps on the basis of making malaria a dispensary disease.

We all know that at about the age of puberty, if the child has lived in an endemic area, a respectable degree of tolerance has been gained but this is not very stable. As soon as these young

TABLE I

*The consolidated annual records for ten years of persons with malaria by age groups*

Chagres River villages. Permanent and Migratory inhabitants (6)

AGE GROUPS	DRUG CONTROL		
	Number examined	Number positive	Per cent positive
0-5	1300	352	27.1
5-10	785	350	44.6
10-20	1330	567	42.6
20-40	1666	452	27.1
40-60	788	206	26.1
Over 60	262	61	23.6
Totals.....	6,131	1,988	32.4

TABLE II

*Rio Pescado control towns. Chagres river-lake shore*

AGE GROUPS	ORIGINAL SURVEY			VOLUNTARY USE OF QUININE, 12 CUMULATIVE MONTHLY SURVEYS		
	Number examined	Positive for malaria	Per cent positive	Number examined	Positive for malaria	Per cent positive
0-5	34	21	61.7	50	28	56.0
5-10	30	26	86.6	62	34	54.8
10-20	22	16	77.2	59	27	45.7
20-40	25	14	56.2	49	15	30.6
40-60	15	6	40.2	29	4	13.8
Over 60	2	1	50.2	3	1	33.3
Totals....	128	84	65.2	252	109	43.2

people enter an active life the fatigue, strain and exposure upset their relatively low degree of immunity. The actual peak of relative immunity would appear to be more accurately measured by what happens to the adolescent after entrance into active business life. I will attempt to demonstrate this by our experience over many years with the Chagres River villages and with a negro labor force from Haiti. These people are strongly negroid in race and have spent their lives

in uncontrolled endemic areas. The former group has been under antimalarial drug control following frequent blood film surveys for the last several years. These groups by race and life-long exposure represent people of high tolerance.

Table I shows in the annual cumulative records that there is a significant incidence of malaria in all age groups, even those above 60 years of age. The peak, however, is shown in the groups of 5 to 20 years.

There is no evidence in Table II of a stable degree of immunity until somewhere between the 20 and 30 years period. Some people never gain a sufficient degree to become parasite-free or symptom-free.

TABLE III

*Men and children in Haiti, 1927 (7)*

	NUMBER EXAMINED	NUMBER POSITIVE	PER CENT POSITIVE
Adults.....	11,000	2585	23.5
Children (2-12 yrs.).....	1,102	462	41.9

TABLE IV

*Degrees of infestation*

	ADULTS	CHILDREN
Heavy infestations.....	25.2	11.9
Medium infestations.....	34.0	44.4
Light infestations.....	40.8	43.7

In 1927 I had an opportunity to make a medical selection of laborers (7) in Haiti for use in the sugar plantations of northeastern Cuba. We were supplied with good technical assistance to do, among other things, a thick blood film survey for malaria. These negroes are as pure descendants of the African negro as can be found in the Caribbean region and therefore could be expected to possess a significant degree of immunity. These men came from all of the civil districts of Haiti. They worked 6 months in the sugar cane harvest of Cuba and then returned to Haiti. No women or children were in the labor camps. However, in order to compare the children's incidence of malaria with the young male labor force a representative number of children from all the civil districts were examined. Tables III and IV show the results of these blood film results.

One year prior to these surveys I was in Cuba at the end of crop which means that the Haitian labor force had been actively employed for 6 months in the field and living in camps. No antimalarial measures were then in force other than hospital and dispensary attention. A similar blood film survey made near the end of crop revealed a parasite index of 66 per cent. A second survey was conducted on men found in camp during working hours and a similar number of men busy cutting cane. The first group gave a rate of 88 per cent and the second 12 per cent. This highly tolerant negro labor force did show a sufficient reaction to malaria, after a lifelong exposure to it, to decrease their labor efficiency. The hospitals received many of them and there were some deaths. However, they need but little help in addition to their relative degree of immunity to remain on duty. For 8 years these men never exceeded 0.9 to 0.93 tons of cut cane per day, per man, per crop. Labor selection and a field program of malaria control during the next three years brought their efficiency up to 1.5 tons. A labor force that has grown up in a rural endemic region without medical or sanitary care is in no position to realize what good health means. The so called "camp loafers" in the sugar cane fields no longer existed in a significant number after malaria control was well established.

A few years after we started antimalarial drug control in the selected Chagres villages, the inhabitants were nearly always able to tell us whether we would find parasites in their blood films.

The peak of relative immunity, 12 to 15 years, that is usually recorded is probably correct for inactive people who have spent their lives in an endemic region but from the viewpoint of an efficient labor force the period from 15 to 30 years requires almost as much attention as the period under 15 years. There is no such thing as absolute

immunity to malaria for the general run of people regardless of race and a long life in malarious regions. Fatigue, exposure, underfed people, introduction of new strains, etc. can break any degree of tolerance and the level of labor efficiency will drop to an important degree.

Non-immunes who enter and remain in such endemic regions will, in most instances, require a very long period of time to acquire a respectable degree of immunity. This will become better recognized by us when our young men return after the war.

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# THE QUININE INHIBITION OF BACTERIAL LUMINESCENCE<sup>1</sup>

FRANK H. JOHNSON AND LEON SCHNEYER

*Microbiological Laboratory, Princeton University, Princeton, N. J.*

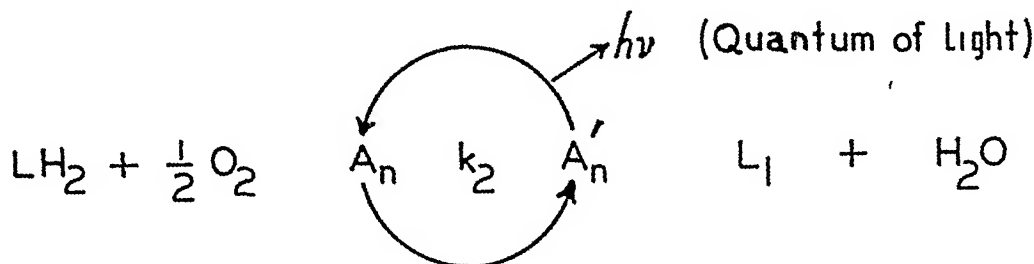
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Although the cinchona alkaloids have been administered for many years as a specific for malaria, no clear understanding has been achieved as to how they accomplish their therapeutic effect. The weight of available evidence indicates that these alkaloids, like various other chemotherapeutic compounds, act directly on the parasite (8, 22, 23, 24). Since such action involves an inhibition of those enzyme reactions essential to the reproduction or viability of the organism, a chemical combination between the drug and some protoplasmic constituent of the pathogen is essential in these reactions.

Recent advances in the theory of enzyme inhibitions and the control of biological reaction rates have made it possible to interpret with some pre-

emitting oxidative enzyme system of luminous bacteria is particularly well suited for this purpose since previous studies with it have provided a satisfactory theoretical basis for the action of typical inhibitors, such as sulfanilamide and urethane.

The luminescent reaction consists of a specific oxidation—in the presence of molecular oxygen—of a substrate, *luciferin*, by an enzyme, *luciferase*. Part of the energy of the reaction is consumed in an excitation of the enzyme molecule, which then radiates on returning to the normal state (9, 10, 11, 12, 13). The cycle of excitation and radiation of the luciferase molecule as it catalyzes the oxidation of the substrate may be represented schematically as follows:



Luciferin    Oxygen    Normal    Excited    Oxidized    Water  
 Luciferase    Luciferase    Luciferin

cision the effects of a given drug, or mixtures of drugs, upon a given enzyme system, in relation to concentration, to temperature, and to hydrostatic pressure; thus revealing the fundamental mechanism of action (2, 7, 16, 17, 18, 19, 20). The purpose of the present investigation has been to extend this type of analysis to include the inhibition of bacterial luminescence by quinine. The light-

where  $k_2$  is the rate constant of the reaction, the prime (') denotes an excited molecule, and the other symbols for substrate, etc., are as indicated. A more precise formulation involving certain intermediate stages has been specified (21). As indicated by the diagram, the intensity of the light is directly related to the over-all velocity of the reaction. Since it has been shown that the slowest, or pace-setting, reaction of the intermediate stages is the combination between luciferin and luciferase (4), the velocity ( $I$ ) of the reaction, i.e., luminescence intensity, is given by the following equation (7):

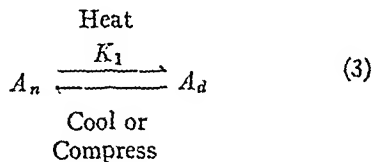
$$I = bk_2(\text{LH}_2)(\text{A}_n) \quad (2)$$

<sup>1</sup> This investigation was carried on in conjunction with a project at New York University concerning the action of the cinchona alkaloids, directed by Dr. Dugald Brown and aided by a grant from the Cinchona Products Institute Inc.

Here  $b$  is merely a proportionality constant,  $k_2$  is again the rate constant, while  $(LH_2)$  and  $(A_n)$  represent the concentration of substrate and active enzyme, respectively. As noted above, the symbol  $(I)$  may therefore stand for either reaction velocity or luminescence intensity. Since luminescence may be easily and accurately measured, an instantaneous index to reaction velocity is readily obtained. It is because of this fact that bacterial luminescence provides such an efficient and uniquely advantageous system for the investigation of quinine inhibitions.

Considering the factors in Equation (2) which may influence the velocity of the reaction, it is obvious that, if the concentration of the reactants remains constant,  $k_2$  will determine the reaction velocity. It has been shown that  $k_2$  varies with temperature and pressure in accordance with the theory of absolute reaction rates (2, 5, 6, 7, 28). Among the reactants the concentration of active enzyme  $A_n$  is of prime significance since, under the experimental conditions, the other reactants may be considered constant. Under any conditions the amount of  $A_n$  is governed by two fundamental influences. The first of these is an equilibrium which normally exists between the native, active form of the enzyme and a denatured, inactive form ( $A_d$ ) of the enzyme (2, 16). The second important factor controlling the amount of  $A_n$  consists in various substances, whether normal metabolic products or added chemical compounds, which combine with the enzyme. The latter category includes drugs, such as sulfanilamide and urethane, whose action can be analyzed satisfactorily only with due regard to the reversible denaturation equilibrium (7, 17, 18, 20).

Diagrammatically, the equilibrium between native and denatured enzyme may be expressed:

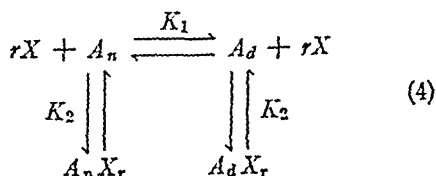


in which  $A_d$  represents the reversibly denatured form of the enzyme and  $K_1$  the equilibrium constant. In luminescence, the equilibrium  $K_1$  is affected by temperature in the same manner as in certain preparations of crystalline enzymes which undergo a reversible temperature inactivation characterized by a high temperature coefficient, upwards of 70,000 calories. The high tempera-

ture coefficient for the equilibrium  $K_1$  is responsible for the rapid reduction in luminescence at the high temperatures. The optimum temperature occurs at that point where the influence of  $K_1$ , governing the amount of enzyme, is just balanced by  $k_2$ , which is controlling the rate of the reaction.

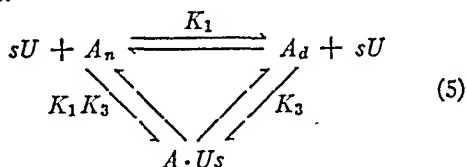
The shift in equilibrium from  $A_n$  to  $A_d$  is also accompanied by a large change in volume (amounting to some 116 cc. per gram molecule) (2) resembling the volume changes of myosin sol-gel equilibria (3) and certain physiological processes (25). As a consequence, the equilibrium is extremely sensitive to hydrostatic pressure. Thus, at above-optimum temperatures where the value of the equilibrium constant  $K_1$  is large, the velocity of the light-emitting reaction is greatly reduced because of the shift of equilibrium in favor of  $A_d$ . Compression will counteract the volume increase involved in this change and shift the equilibrium back in favor of  $A_n$ .

With regard to the action of drugs, the equilibrium  $K_1$  is of key significance. For it follows from the diagram (3) above, and it has been shown with various reversible inhibitions, that two distinct types of combinations with the enzyme are possible, either of which may reduce the amount of active catalyst and thereby inhibit the reaction (20, 26). The type differs according to whether the equilibrium established is independent of the denaturation equilibrium  $K_1$  (Type I) or is directly related to it (Type II). In Type I, the drug or inhibitor combines equally with  $A_n$  and  $A_d$  at chemical bonds not involved in the reversible denaturation, much as if the combination took place with, or in place of, a prosthetic group. Diagrammatically, this type may be represented as follows, in which  $X$  represents a given concentration of the inhibitor,  $r$  the number of molecules of  $X$  combining with each enzyme molecule, and  $K_2$  the equilibrium constant for the enzyme-inhibitor combination:



In contrast to the above, if a substance in concentration ( $U$ ) combines in an equilibrium ( $K_3$ ) with the enzyme at bonds made available in its thermal denaturation, it will promote the reversible

temperature inactivation. In this event, the inhibitor-enzyme equilibrium,  $K_3$ , will be profoundly influenced by the value of  $K_1$ . Diagrammatically, we may represent this type (II), in which  $s$  represents the number of inhibitor molecules combining with each enzyme molecule, as follows:



Alcohols, urethane, and other protein-denaturing substances illustrate Type II in the luminescent system, whereas sulfanilamide illustrates Type I.

In order to distinguish between Type I and Type II, it is necessary to analyze the temperature and pressure relations. Although the concentration of the drug is an important variable, the analysis with respect to concentration is the same for both types and reveals simply the ratio of combining molecules,  $r$  or  $s$  respectively. Temperature, however, will in general influence the inhibition caused by the two types in a somewhat different manner. Thus, in Type I, the inhibition is likely to decrease as the temperature is raised, for the enzyme-inhibitor complex becomes dissociated. The optimum, or maximum, shifts to slightly higher temperatures. In Type II, on the other hand, although a rise in temperature will again tend to dissociate the enzyme-inhibitor complex, the increased temperature will be even more effective in making available the chemical bonds with which the inhibitor unites. The net result in this case will be an increase in inhibition as the temperature is raised, and a shift in the optimum, or maximum velocity, to lower temperatures. Furthermore, unlike the first type, Type II inhibition might be expected to be very noticeably sensitive to hydrostatic pressure because of its relation to  $K_1$ , involving large volume changes.

With the general basis set forth above, we may now undertake to analyze the action of quinine in inhibiting the luminescent system. The full analysis will clearly entail experiments to determine the reversibility of the effect, and the relation of drug concentration, of temperature, and of pressure to the amount of inhibition.

#### METHOD

The experiments described in this report deal with the action of quinine on the metabolism of

essentially non-proliferating bacteria. Since detailed descriptions of methods and apparatus have already been given in the papers cited, a few general remarks should suffice here. The organisms were cultivated on the surface of 3% NaCl nutrient agar, containing 1% glycerol and 0.2 to 0.5%  $\text{CaCO}_3$ , at their optimum temperature: 17°C. for *Photobacterium phosphoreum* and 25°C. for *Achromobacter fischeri*. These species have been studied most intensively and are more convenient from an experimental point of view than organisms with optimum temperatures of 37°. The cells of young, brightly luminous cultures were emulsified in a phosphate-buffered salt solution consisting of equal parts of 3% NaCl and M/4 sodium phosphate buffer, pH 7.3, which we have referred to as "PN" for short. The suspension was aerated with a stream of air, and then added to the desired concentrations of quinine dissolved in "PN". The suspension was placed in a water bath at constant temperature, and the intensity of luminescence was measured by a photoelectric cell and DC amplifier, modified from the system described by Shapiro (1934). In the experiments with hydrostatic pressure, the cell suspension was added to various quinine concentrations and introduced into a specially constructed gold plated, high tensile bronze bomb with a herculite window, and luminescence was measured at various pressures applied from a hydraulic pump. The bomb was maintained at a constant temperature in a water bath. A modified Leeds and Northrup MacBeth illuminometer was used in the pressure studies instead of the photo cell.

#### RESULTS OF EXPERIMENTS

##### a. Relation of the quinine inhibition to time; to cell concentration; and reversibility of the inhibition

When quinine is added to the bacterial suspension, luminescence immediately decreases to a lower intensity, which, under favorable conditions of temperature and adequate substrate, then remains constant for a considerable period of time, 30 to 45 minutes. The control, without quinine, likewise remains constant, thus enabling accurate comparisons of the two to be made. The fact that this inhibition does not result from killing of some of the cells or other form of irreparable damage may be clearly shown by centrifuging the organisms and resuspending them in a fresh salt

solution without quinine. In this event, the intensity of luminescence is almost completely restored to the value existing before the inhibitor

hibitory properties, showing that no considerable amount of the inhibitor is removed by combining irreversibly with cell constituents. Moreover, as

TABLE I  
*Reversibility of the quinine inhibition of luminescence*

TUBE	SALT SOLUTION PN	QUININE 0.0015 M IN PN	BACTERIA SUSPENDED IN PN	INTENSITY OF LUMINESCENCE	PROCEDURE	INTENSITY OF LUMINESCENCE	PER CENT INHIBITION	
							Before procedure	After procedure
1	5	5	5	8.2	Centrifuge; resuspend cells in quinine-free PN solution	8.3	50	4
2		5	5	4.0		7.9		
3	5		5	8.1	Dilute with 10 cc. quinine-free PN, mix, then discard 10 cc.	4.2	48	14
4		5	5	4.2		3.6		
5			5		Add 5 cc. supernatant fluid decanted from tube 1	8.6		
6			5		Add 5 cc. supernatant fluid decanted from tube 2	6.3		27

TABLE II  
*Relation of the per cent inhibition of luminescence by 0.00075 M quinine to cell concentration*

TUBE	SALT SOLUTION PN	0.0015 M QUININE IN PN	RELATIVE CELL CONC. BY DILUTION OF SUSPENSION OF BACTERIA	LUMINESCENCE INTENSITY				PER CENT INHIBITION
				Repeated readings			Average	
1	5	5	1.0	8.8	8.9	9.0	8.9	66
2		5	1.0	2.9	2.9	3.1	2.9	
3	5		0.8	8.6	8.2	8.3	8.4	62
4		5	0.8	3.3	3.0	3.2	3.2	
5	5		0.6	6.0	5.4	5.4	5.6	55
6		5	0.6	2.5	2.3	2.8	2.5	
7	5		0.4	4.2	4.2	3.8	4.1	63
8		5	0.4	1.5	1.5	1.4	1.5	
9	5		0.2	1.9	1.9	1.9	1.9	58
10		5	0.2	1.0	0.7	0.8	0.8	
11	5		0.1	0.9	1.0	1.0	1.0	50
12		5	0.1	0.5	0.5	0.5	0.5	
13	5		0.05	0.6	0.7	0.7	0.7	57
14		5	0.05	0.3	0.3	0.4	0.3	

was added. Similarly, the per cent inhibition is readily decreased simply by dilution. The supernatant fluid, after the removal of the cells from the quinine solution by centrifuging, retains its in-

would be expected, the inhibition caused by a given concentration is practically independent of cell concentration. These facts are demonstrated by the data in Tables I and II. They indicate

that the inhibitory action of quinine on luminescence, like that of urethane, sulfanilamide, alcohols, and other drugs recently studied, involves a reversible combination, or equilibrium, between the drug and one or more catalytic systems involved in the light-emitting reaction. The theory developed for the inhibitions studied earlier, therefore, applies in this case also, and provides a basis for further analysis.

*b. Relation of the inhibition to concentration of quinine*

The analysis proceeds as follows. Omitting the derivations which have already been set forth at length in earlier publications (20), we arrive at the following equations, in which  $I_1$  represents the luminescent intensity or velocity of the enzyme reaction in the control,  $I_2$  the intensity or velocity in the presence of added inhibitor, and the other symbols— $X$ ,  $U$ ,  $r$ ,  $s$ ,  $K_2$ , and  $K_3$ —have the same meaning as before:

For Type I

$$\left(\frac{I_1}{I_2} - 1\right) = K_2 X^r \quad (6)$$

For Type II

$$\left(\frac{I_1}{I_2} - 1\right)\left(1 + \frac{1}{K_1}\right) = K_3 U^s \quad (7)$$

From these equations it is apparent that, if the temperature and pressure remain constant, the values of  $K_1$ ,  $K_2$ , and  $K_3$  will remain constant. If the concentration of  $X$  or  $U$  is varied, however, a straight line should result when the  $\log. \left(\frac{I_1}{I_2} - 1\right)$  is plotted against the logarithm of molar concentrations of drug. The slope of this line gives the value of  $r$  or  $s$ , without distinguishing which, and represents the ratio of drug to enzyme molecules in that equilibrium combination. If the line is not straight, it indicates that the drug combines with more than one substance (18).

The relation between quinine concentration and the amount of inhibition of luminescence for *P. phosphoreum* at three temperatures is shown in figure 1. At the optimum temperature (22°C.) the line appears to be slightly curved, indicating that the drug combines at more than one site. In repeated experiments, such a departure from a straight line relation occurred to different extents, and in some cases an actual stimulation of luminescence was observed with very low concen-

trations of the drug, possibly through a mechanism similar to that discussed earlier (18). In general, however, the lines in concentration plots have been sufficiently straight to make possible an estimation of the ratio of molecules in the quinine-enzyme combination. In figure 1, the slope, and hence this ratio, is approximately 1.3. In repeated experiments the slope varied between 1 and 1.5.

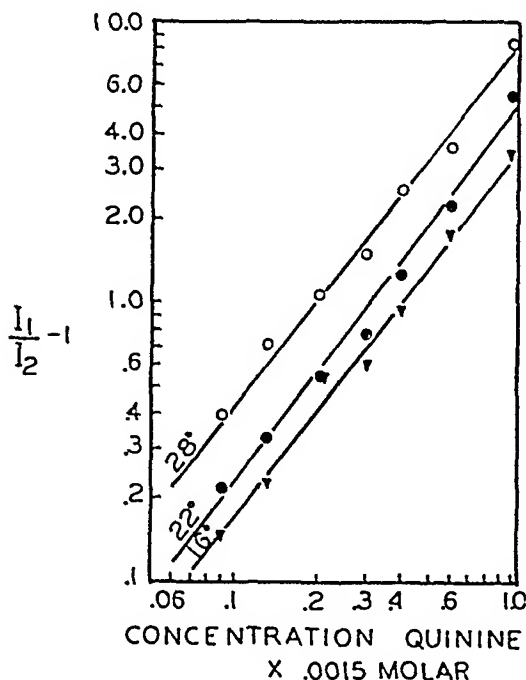


FIG. 1. RELATION BETWEEN CONCENTRATION OF QUININE AND INHIBITION OF LUMINESCENCE IN *P. PHOSPHOREUM* AT 16°, 22°, AND 28°C., RESPECTIVELY, PLOTTED ON A LOG-LOG SCALE IN ACCORDANCE WITH THE METHOD OF ANALYSIS REFERRED TO IN THE TEXT

*c. Relation of the inhibition to temperature and pressure*

With regard to quinine, figure 2 shows the relation between luminescence intensity and temperature for corresponding suspensions of cells without added inhibitors, and with two concentrations of quinine, 0.0003 M and 0.0006 M, respectively. A slight increase in sensitivity to the inhibitor is apparent as a result of prolonged aeration of the suspension before adding the drug. In general, the inhibition increases with a rise in temperature, and the normal optimum becomes increasingly shifted to lower temperatures with increasing concentrations of the drug. These relations strongly suggest that quinine acts as a Type II inhibitor.

In figure 3 the results are shown for two different species with different normal temperature optima. This figure illustrates several points of fundamental significance. In the first place, the general relationship of the inhibition to temperature, with strict reference to the normal temperature curve of the species rather than the absolute temperature concerned, is the same in both. Thus, a given

effectiveness may be accounted for, in the first instance, on the basis of the difference in the normal temperature-intensity relation of a given process in two different species. Thus, a 35% inhibition of luminescence in *P. phosphoreum* occurs at 15°C. In *A. fischeri*, the same concentration, although giving rise to only a 22% i 15°C., will cause a 35% inhibition if t

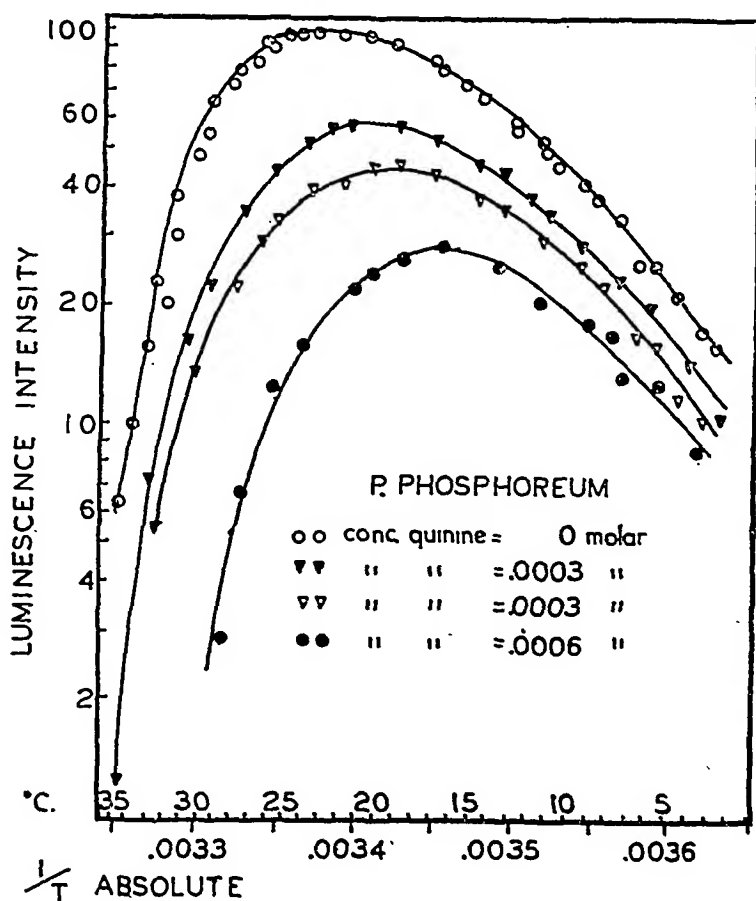


FIG. 2. RELATION BETWEEN TEMPERATURE AND INTENSITY OF LUMINESCENCE OF *P. PHOSPHOREUM* WITH AND WITHOUT ADDED QUININE

Luminescence expressed in arbitrary units, with the maximum for the control taken at 100. Long aeration of the cells in buffered salt solution caused a slightly greater sensitivity to 0.0003 M quinine. Hollow triangles represent measurements about two hours later than those shown by solid triangles. Note the increase in inhibition with rise in temperature.

concentration of quinine, such as .00038 M, acting upon *P. phosphoreum* causes an inhibition that varies from approximately 35% at 15°, to 65%—or nearly double—at 25°C. At 30°, the inhibition increases to 76%. With a different species, *A. fischeri*, which has a higher optimum temperature, the same concentration of quinine causes less inhibition at the same temperatures: 22% at 15°, 32% at 25°, and 35% at 30°. The difference in

ture is raised to 30°C. At any one temperature, and with either organism, of course, the inhibition may be increased by increasing the concentration of quinine, in the manner illustrated in figure 1.

The influence of temperature on the degree of inhibition at any given concentration of quinine depends upon whether the drug is acting as a Type I or Type II inhibitor. Provided that the action is on a single system in the manner specified in

Equations (6) and (7), the above data yield the values of  $K_1$  and  $K_3$  necessary to distinguish which type of action is represented. In proceeding with the analysis, it may be recognized that the equilibrium constant  $K_1$ ,  $K_2$ , or  $K_3$  may be defined thermodynamically, as usual, using the subscripts to

heat of reaction ( $\Delta H_1$ ) or temperature coefficient, amounting to upwards of 70,000 calories. (At ordinary pressures  $\Delta H$  is indistinguishable from the familiar  $\mu$ ). The values of either  $K_1$ ,  $K_2$ , or  $K_3$  in Equations (6) and (7) may be expressed in terms of the equivalents given in Equation (8).

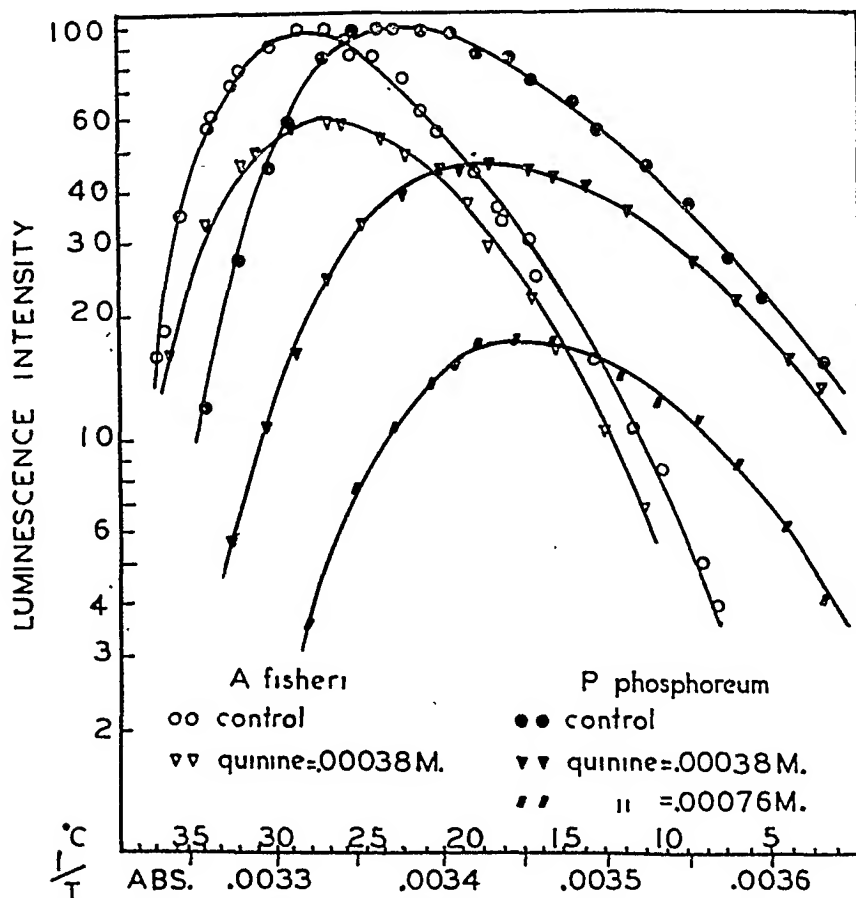


FIG. 3. INTENSITY OF LUMINESCENCE OF TWO SPECIES OF BACTERIA IN RELATION TO TEMPERATURE AND QUININE

denote which of the three equilibria is concerned in a given case (e.g.,  $K_1$ ):

$$K_1 = c^{-\frac{\Delta F_1}{RT}} = c^{-\frac{\Delta H_1}{RT}} c^{\frac{\Delta S_1}{R}} = c^{-\frac{\Delta E_1}{RT}} c^{-\frac{p\Delta V_1}{RT}} c^{\frac{\Delta S_1}{R}} \quad (8)$$

in which  $\Delta F$  is the free energy change,  $\Delta H$  is the heat of reaction,  $\Delta E$  is the energy of reaction,  $\Delta V$  is the volume change of reaction, and  $\Delta S$  is the entropy of reaction; with  $R$  the gas constant and  $T$  the absolute temperature. The value of  $K_1$ , needed in Equation (6), may be obtained from the normal curve relating velocity or intensity to temperature. In the cases studied, this has a high

From the point of view of analysis, this means that, for Type I, if the logarithm of  $\left(\frac{I_1}{I_2} - 1\right)$ , for a given concentration of inhibitor compared with the control, is plotted against the reciprocal of the absolute temperature, the slope of the line gives the value of  $\Delta H_2$ , and the intercept gives the entropy,  $\Delta S_2$ . If the line is not straight, it means that the drug either inhibits more than one reaction, inhibits the observed reaction indirectly, or does not conform to Type I. Conformity to Type II is tested by plotting the logarithm of



$\left[\left(\frac{I_1}{I_2} - 1\right)\left(1 + \frac{1}{K_1}\right)\right]$  against  $\frac{1}{T}$ . The slope of the line gives the value of  $\Delta H_3$ , and the intercept the entropy,  $\Delta S_3$ .

Although anticipating the results of compression, it may be pointed out that the volume change,  $\Delta V_3$ , may be obtained as a first approximation from the slope of the line, which may or may not be straight, when the logarithm of  $\left(\frac{I_1}{I_2} - 1\right)$  is plotted against pressure. For Type II inhibitors, the  $\log. \left[\left(\frac{I_1}{I_2} - 1\right)\left(1 + \frac{1}{K_1}\right)\right]$  should be plotted against pressure, but the values of  $K_1$  in this case involve laborious calculations.

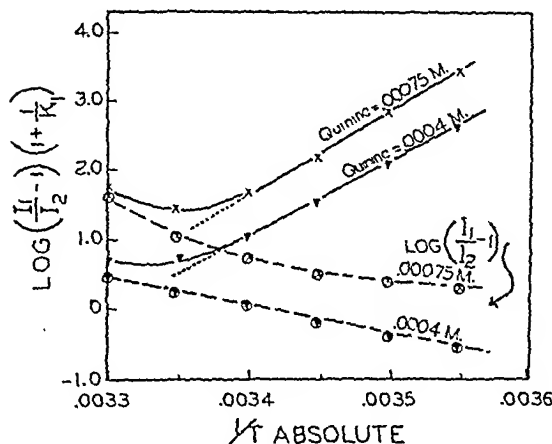


FIG. 4. ANALYSIS OF THE DATA IN FIG. 3, ACCORDING TO THE FORMULATIONS FOR TYPE I AND TYPE II INHIBITORS, RESPECTIVELY

In figure 4 are presented the data for *P. phosphoreum*, as given in figure 3, analyzed both as a Type I and a Type II inhibitor. It is evident that the effect of quinine is somewhat more complicated than that of sulfanilamide or urethane, whose analysis, according to the appropriate Type, yields in each case a straight line over a wide range of temperatures (18, 20). That a direct action of quinine on the luminescent enzyme system occurs is shown by the inhibition of luminescence in crude extracts of *Cypridina*. In bacteria, possibly an antecedent reaction is also affected. In Fig. 4 the results are more in accord with expectations for a Type II than a Type I inhibition, and on this basis the departure from a straight-line relation at above-optimum temperatures might be interpreted either as a partial destruction of the reversibly inhibited enzyme of the luminescent system, or as an additional inhibition of an antecedent reaction. The

available data and formulations are not sufficient for final analysis at present. Further evidence, however, concerning its mode of action, is provided by data on pressure effects and mixtures with other inhibitors, as set forth in the following paragraphs.

#### d. Pressure and luminescence

The influence of pressure at different temperatures on the observed intensity of luminescence, both without added inhibitor and in the presence of 0.00038 M quinine, is shown in figure 5. At each temperature, the control—without quinine and at normal pressure—has been arbitrarily taken as 100, in order to show more clearly the per cent differences. The change in pressure effect at different temperatures, both with respect to the control and the quinine-inhibited suspensions, is strikingly apparent. When these data are analyzed according to the formulations referred to above, the series of practically parallel and straight lines shown in figure 6 results. The slope of these lines indicates that the net volume change in the combination of quinine and enzyme is relatively large, amounting to about 20 cc. per gram molecule. This value must be considered as only a first approximation, however, because more than one system contributing to luminescence is probably affected. Furthermore, as indicated above, if the quinine acts as a Type II inhibitor in all cases, the value of  $K_1$  should be taken into account in calculating the volume change under a given set of conditions.

In figure 7 the effects of pressure, at a constant temperature near the optimum, are analyzed for various concentrations of quinine. The slopes of the lines again indicate a volume change of about the same amount, varying somewhat with concentration and pressure. The changes in slope evident in figure 7, both with concentration and with pressure, might reasonably be expected in view of the considerations already expressed. Quantitative predictions regarding these slopes on purely theoretical grounds are not possible.

The significance of the pressure data rests largely in two facts: first, that a more complete physicochemical interpretation concerning the fundamental nature of the quinine inhibition is provided; second, that the action of quinine in luminescence is shown to resemble that of various Type II inhibitors which act by promoting the reversible denaturation of the protein in the enzyme prima-

rily affected. Thus, although the temperature studies revealed a complexity of action that cannot be analyzed in the manner of a simple Type I or Type II inhibitor acting only on the light-emitting system directly, the pressure studies, as well as other considerations, make it clear that, in

hols (Type II), would evidence a wide degree of both synergism and antagonism, depending on temperature, relative concentrations, and the types of drugs mixed. Type I and Type II inhibitors might be expected to combine with each other as well as with the enzyme, possibly by hy-

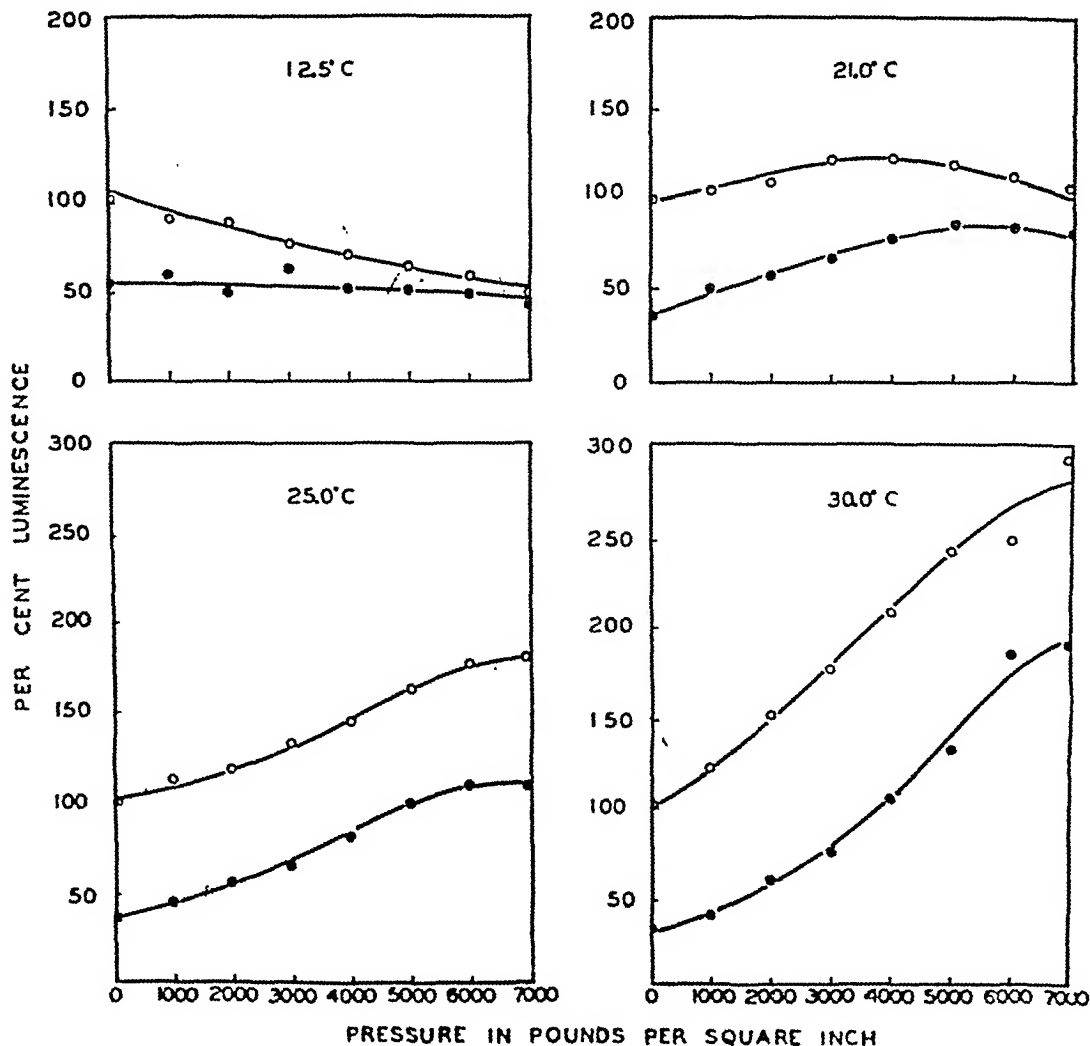


FIG. 5. INTENSITY OF LUMINESCENCE IN *P. PHOSPHOREUM* WITH AND WITHOUT QUININE, IN RELATION TO HYDROSTATIC PRESSURE, AT DIFFERENT TEMPERATURES

In each case, the control at normal pressure has been arbitrarily taken as 100, and all intensities calculated on this basis.

general, quinine acts on this process very much in the manner of a Type II inhibitor.

#### c. Mixtures of quinine with other inhibitors

From the previous studies (18) it would be predicted that mixtures of quinine with other inhibitors, such as sulfanilamide (Type I) or alco-

drogen bonds. The effective inhibitory concentration is thus reduced, with the result that a greater or less antagonistic action is noted according to the value of the equilibrium constants concerned. On the other hand, two inhibitors of the same type may be expected to show mostly synergistic effects with a wide range of effective con-

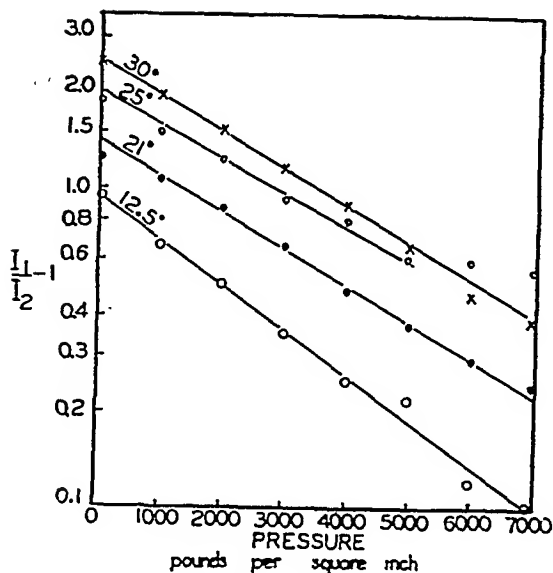


FIG. 6. ANALYSIS OF THE DATA GIVEN IN FIG. 5 ACCORDING TO THE FORMULATIONS REFERRED TO IN THE TEXT FOR ESTIMATING THE MOLECULAR VOLUME CHANGE IN THE QUININE-ENZYME EQUILIBRIUM

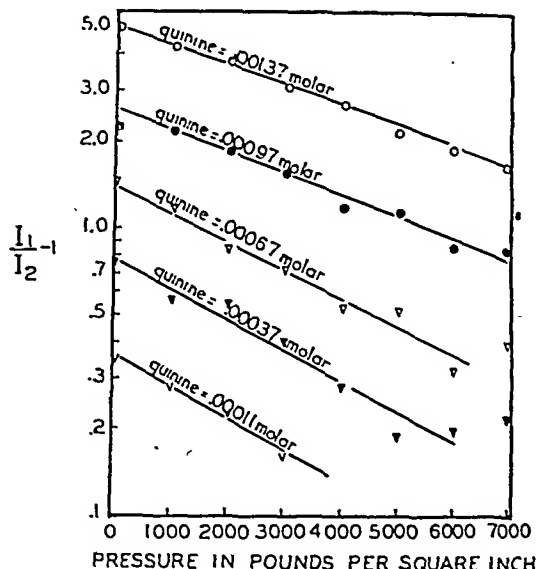


FIG. 7. RELATION BETWEEN INHIBITION OF LUMINESCENCE OF *P. PHOSPHOREUM* AND CONCENTRATION OF QUININE AT 20.1°C., AND DIFFERENT HYDROSTATIC PRESSURES

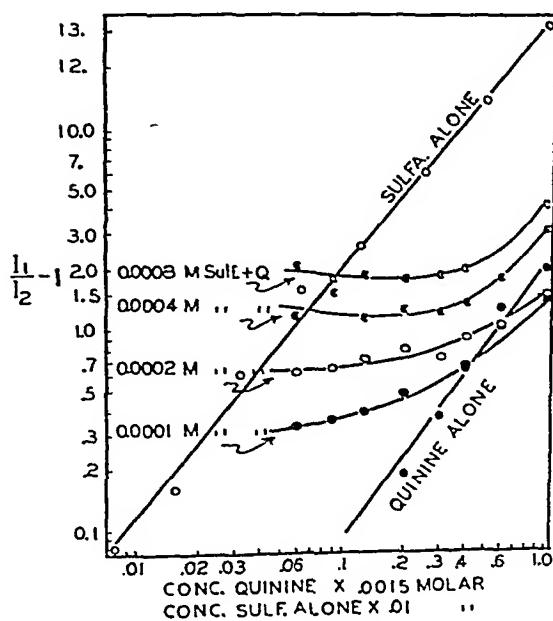


FIG. 8. THE INHIBITION OF LUMINESCENCE IN *P. PHOSPHOREUM* BY SULFANILAMIDE ALONE, QUININE ALONE, AND VARYING CONCENTRATIONS OF QUININE WITH DIFFERENT CONSTANT CONCENTRATIONS OF SULFANILAMIDE, RESPECTIVELY  
Log-log scale

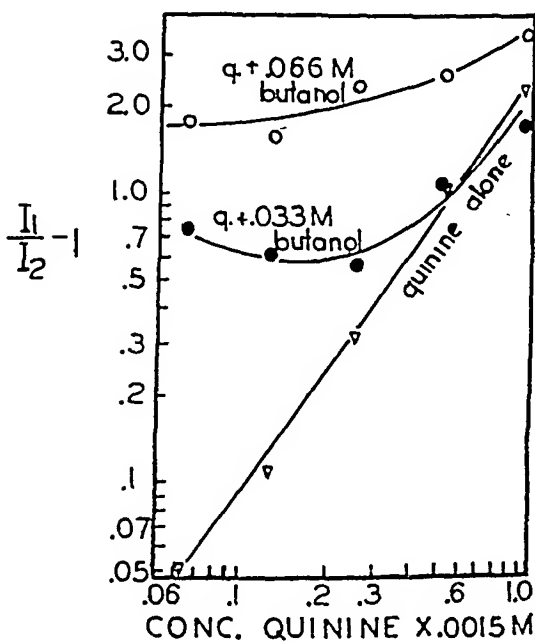


FIG. 9. THE INHIBITION OF LUMINESCENCE IN *P. PHOSPHOREUM* BY SULFANILAMIDE, QUININE, MIXTURES OF QUININE AND CONSTANT 0.003 M SULFANILAMIDE, AND MIXTURES OF QUININE WITH TWO DIFFERENT CONSTANT CONCENTRATIONS OF BUTYL ALCOHOL, RESPECTIVELY  
Log-log scale

centrations. The theoretical basis for these effects has been set forth in detail with respect to sulfanilamide plus alcohols, ether, urethane, chloroform, and acetone. The analysis has not been worked out in final, quantitative form in the present study.

In regard to quinine, qualitative theoretical predictions have been realized, as shown in figures 8 and 9. For example, in figure 8, with luminescence moderately inhibited by sulfanilamide, little or no increase in total inhibition occurs on adding quinine. With higher concentrations of sulfanilamide causing greater initial effects on luminescence, the addition of small amounts of quinine actually causes a decrease in inhibition, i.e., antagonizes the sulfanilamide action. Conversely, with the low sulfanilamide concentrations, the effect of high quinine concentrations becomes less and the action of quinine may be considered antagonized by sulfanilamide. Similar relations are apparent in figure 9, which shows further that butyl alcohol inhibition may also be antagonized by quinine, and vice versa. The latter point is interesting, for by antagonizing both sulfanilamide and an alcohol, quinine to some extent displays the properties of both types of inhibitors—a possibility consistent with the temperature data given above.

In all cases it is especially to be noted that the presence of a constant amount of one inhibitor profoundly alters the physiological effect of adding increasing concentrations of a second inhibitor. The departure from a straight line relation in the plot of  $\log. \left( \frac{I_1}{I_2} - 1 \right)$  against  $\log.$  concentration of quinine added alone, as discussed earlier, may be due to equilibria established between the quinine and normal metabolites formed by the organisms themselves and already limiting the luminescent reaction. Apparent "stimulations" might easily arise in this manner, and it would seem likely that mild stimulations of various processes by small concentrations of diverse poisons frequently involve such a mechanism. It may be added that this phenomenon of loose combination, or complex formation between two physiologically active drugs, has been shown by alterations in solubilities to take place independently of biological systems. It would, therefore, undoubtedly occur in the body, although the complexity of higher animals with numerous compensating mechanisms might easily obscure the results that would be expected for simple systems.

#### DISCUSSION

The results described in this investigation are interesting primarily because they provide fairly complete data with regard to the fundamental action of quinine on a single physiological process, luminescence, in living bacteria. Among the relations thus established, the following appear worthy of note.

The observed quinine action is fundamentally similar to that of a large group of substances that act to promote a reversible denaturation on the protein portion of enzymes. Since the equilibrium which normally governs the reversible denaturation is sensitive to heat and pressure, the degree of inhibition by certain drugs is also affected by heat and pressure. It is not surprising that the data indicate that quinine acts on more than one stage in the reaction leading to luminescence. Such drugs might be expected to act on various enzymes, though obviously not equally, for the actual inhibition obtained will depend to a considerable extent on the value of the normal reversible denaturation equilibrium,  $K_1$ . This equilibrium will differ intrinsically among different proteins, and may also be influenced by a number of environmental factors, such as heat, pressure, hydrogen ion concentration, et cetera. From another point of view, it may be said that the effect of quinine, under given conditions, will depend on the optimum temperature of the process in question. Except in so far as this drug behaves like a Type I, instead of the typical Type II inhibitor, a rise in temperature will accentuate its action.

The temperature relation is of such fundamental importance that it perhaps justifies a speculation concerning the mechanism of quinine therapy. By analogy with other chemotherapeutic compounds whose mode of action has been so extensively investigated of late (8, 23, 24), as well as some more direct evidence (14, 22), one might suppose that the success of the drug depends primarily on a direct effect on viability or an inhibition in the rate of reproduction of the parasite within the body of the host; thereby enabling the normal defense mechanisms of the latter to eliminate the invader. The effect in either case—on immediate viability or on rate of reproduction—will be inevitably related to the specific optimum temperature of the rate-controlling reaction, or reactions, affected. While the medical action of the drug must take place within the rather narrow temperature limits imposed by the host, i.e., ap-

proximately 37 and 42°C., *a priori* there is no reason to suppose that the optimum temperature for processes in the parasite affected by the drug will coincide necessarily with that of the host. If the optimum temperatures of such processes differ from those of the host, a possible explanation for differences in the effectiveness of quinine for different types of malarial organisms is at once apparent, purely on the basis of relatively slight differences in optimum temperatures of the different parasitic species. Such differences, arising from both genetic and environmental causes, are one of the most frequently encountered types of biological variation.

On the basis of the same interpretations, if the optimum temperatures of metabolic reactions in the host are, in general, above the optimum for those in the parasite, a greater sensitivity of the latter to the drug would result, even if the quinine combined according to exactly the same equilibrium constant ( $K_2$ ) in all cases. Seen in this light, fever or Type II compounds, such as alcohols, might be expected to increase the effectiveness of a given concentration of quinine, except in so far as the normal processes of the host are themselves made more susceptible to injury by the unfavorable temperatures and additional drugs. The success of increased bodily temperature by diathermy in aiding the arsphenamide treatment of protozoan infections, such as syphilis, quite possibly rests on the same rational principle. It suggests that application of such methods in other cases should be worth further investigation, particularly if the type of drug action can be identified as one which is accentuated by a rise in temperature.

Finally, it should perhaps be emphasized that the ultimate elucidation of the specific quinine therapy in malaria must of necessity be considered in terms of a chemical reaction. The specificity of its action is a quantitative problem, for the drug clearly enters into the formation of a number of compounds or complexes. The problem involves learning the comparative readiness, as well as types of substances, with which quinine combines. The theoretical formulations applied in this paper provide a reliable basis for the comparison and analysis of specific reactions of quinine and other anti-malarials.

#### SUMMARY

The intensity of luminescence in non-proliferating, luminous bacteria suspended in phosphate-

buffered salt solution is immediately decreased on addition of quinine. This inhibition is independent of cell concentration and is immediately reversible by diluting or by centrifuging and re-suspending the cells in a medium without quinine.

An analysis of the relation between concentration and inhibition, at constant temperature and hydrostatic pressure, indicates that the average ratio of combining molecules is 1 to 1.5 for quinine and its site of action, respectively, in the equilibrium.

The relation of inhibition to temperature shows that it is dependent on the specific, normal, optimum temperature of the process in the organism concerned. In general, the inhibition increases as the temperature is raised towards and beyond the optimum. A given concentration of quinine, at a given temperature, may thus result in widely different potencies of effect according to the normal temperature-intensity relation of the luminescent oxidative reaction in different species. In the presence of the drug, the optimum is shifted to lower temperatures than normal.

Experiments with crude extracts of the luminescent luciferin-luciferase system of *Cypridina* show that the luminescent reaction may be directly affected by quinine. In bacteria, an analysis of the temperature data indicates that more than a single reaction is affected. In general, however, the type of action resembles that of urethane, alcohols, acetone, and similar inhibitors which promote reversible denaturation of the protein enzyme.

The quinine inhibition of bacterial luminescence is sensitive to hydrostatic pressure, which, under certain conditions, may completely abolish the effect of the drug.

Evidence for a combination of quinine with sulfanilamide and with butyl alcohol, predictable on theoretical considerations, was found in the mutual antagonism of the luminescence inhibition exhibited by mixtures of these drugs at favorable concentrations and temperatures. In general, the net result obtained with mixtures was either an antagonism or synergism, depending on the relative concentrations involved and the tendency of the drugs to combine with each other as well as with the catalytic systems in the cells.

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# THE CYSTICIDAL EFFECTS OF CHLORINE AND OZONE ON CYSTS OF ENDAMOEBIA HISTOLYTICA, TOGETHER WITH A COMPARATIVE STUDY OF SEVERAL ENCYSTMENT MEDIA

JOHN F. KESSEL, DONALD K. ALLISON, MARTHA KAIME, MARIA QUIROS, AND  
ALBERT GLOECKNER

*From the Department of Bacteriology and Parasitology, School of Medicine, University of Southern California, and  
Pathology Laboratory, Los Angeles County Hospital<sup>1</sup>*

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## INTRODUCTION

Chlorine has come to be generally accepted for use in water purification procedures during the experiences of the last two or three decades. Ozone also has been known for years to be a highly effective oxidizing agent and has been used extensively in Europe for water sterilization, as well as for the reduction of objectionable tastes and odors. The extensive use of ozone in this country, however, has been relatively retarded because of: 1) the high costs of power in the United States during the period of ozone development, and 2) the low electrical efficiency of the older types of ozone equipment. In recent years the cost of electrical power in this country has been greatly decreased and the efficiency of the ozone<sup>2</sup> system used in this study has been improved to such a degree that ozone can now be produced at costs per pound comparable with chlorine. It should further be pointed out that one pound of ozone is the chemical equivalent of one and one half pounds of chlorine. Thus the economic picture in production shifts strongly in favor of ozone.

While certain of the early work with ozone indicates its bactericidal properties, few quantitative tests have been reported and with the exception of studies in this laboratory no records are known to the authors which compare the bactericidal effects of chlorine and ozone nor the effect of ozone on protozoan cysts or viruses (1).

The effect of chlorine on cysts of *E. histolytica* has been studied by several workers but until recently the results could not be compared because of lack of uniformity in techniques. The earlier work in which culture methods were not employed in tests for viability of cysts were highly specula-

tive and variations in conclusions were marked. Investigations to be of value should compare, by standard methods, the dosage and residual of chlorine in p.p.m., the temperature, pH, and organic nitrogen content, together with cyst and bacterial count per cc. of the water tested.

Among the more recent reports which may be reviewed are those of Liu (2), Garcia (3), Gordon (4), Stone (5), Chang and Fair (6) and Brady, Jones and Newton (7). Even in these reports some difficulty arises in comparing results because of the selected variables in the experiments.

Stone (5) shows, at a temperature of 22 to 25°C., with organic nitrogen of 0.12 p.p.m., with probably 1500 cysts per cc., that .6 p.p.m. residual of chlorine in 60 minutes and 1.6 p.p.m. in 15 minutes will destroy cysts of *E. histolytica*. He concludes that *Escherichia coli* and cysts of *E. histolytica* are about equally susceptible to chlorination though he does not state the bacterial count per cc. of his experiments. He used both calcium hypochlorite (H. T. H.) and chlorine gas but does not differentiate between the effectiveness of the two.

Chang and Fair (6) conclude that the concentration of gaseous chlorine needed to destroy cysts of *E. histolytica* appears to be well within the range of practicable super-chlorination provided the contact period is 30 minutes, or more. They indicate, by most efficient graphs, the effect of such variables as temperature, pH, organic nitrogen and cyst density. No comparison is reported between the bactericidal and cysticidal effects of chlorine under identical conditions.

Brady et al. (7) tested the effect of H. T. H. in the Lyster Bag under field conditions and indicate that for practical purposes it probably requires 7.5 mg. of applied hypochlorite per litre for 15 to 20 minutes to inactivate 99% of 20 cysts of *E. histolytica* per cc. Their initial pH values varied between 6.5 and 7.2, their temperature between

<sup>1</sup> Aided by a grant from the Lane-Wells Company.

<sup>2</sup> The ozone used was produced by a laboratory model of the Sterozone ozonizer, designed by one of the authors, D. K. A.



19 and 28°C., and organic nitrogen between .1 and .65 p.p.m.

#### MATERIALS AND METHODS

For the current study it was suggested by specialists in the armed forces that chlorine, producing residuals of 0.5 p.p.m. and of 1.00 p.p.m. respectively, be compared with ozone for various time intervals at several pH levels. In a series of preliminary tests with unbuffered water having an initial pH ranging between 6 and 7 it was found that ordinarily the application of gaseous chlorine and of ozone lowered the pH while the application of sodium hypochlorite increased the pH of the tested medium. In order to obviate the possible influence of the bactericidal and cysticidal effect of pH it was decided to buffer the original solutions so that changes in pH would not occur during the experiment.  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$  were used in the proper proportions for the pH ranges from 5 to 8 and  $\text{KCl}$ ,  $\text{NaOH}$ , and  $\text{H}_3\text{BO}_3$  were used for the solutions buffered to pH 9.

#### Media

The first problem in a study on cysticidal effects of any substance is to be able to produce cysts in sufficient quantity so that an adequate number of freshly viable cysts will be available for experiments at regular intervals. Several methods have been reported in the literature, i.e., Boeck and Drbohlav's medium with starch as reported first by Dobell (8), 2) Cleveland's medium (9), 3) Stone's medium (10), and 4) Chang's medium (11), and each has produced sporadic results in the hands of the present authors. Since Chang<sup>3</sup> (11) reported excellent results by using a highly buffered liver extract liquid medium one of the authors in the current work (M. Q.) suggested that a buffered solid base might produce even more stable results than a buffered liquid medium. Both Difco's Liver Infusion Agar, and R. E. S. were buffered by the same phosphate buffer used by Chang and the serum used over the slant was likewise diluted with this buffer, which produced a medium at pH 7 to 7.2 of M/27 phosphate containing 0.9% chloride salts. The buffered liver infusion agar produced more cysts than the buffered egg slant, hence this was selected for routine work.

Chang's medium contains an excess of starch

which is routinely filtered off before cysts are used. By quantitative standardization of the starch added to the buffered liver infusion agar encystment medium it is possible to procure cysts with a minimum of residual starch. In this study 0.01 mg. of starch is added to each tube containing 8-10 cc. of 1:10 Serum-Ringer's solution placed over the slant.

In general our procedure is as follows:

1. Grow the trophozoites 48 hours in small bottles containing Cleveland and Collier's Difco medium 2% agar without starch. The equivalent of three 17 x 150 mm. test tubes is placed in one bottle.

2. Pool the sediment containing the trophozoites from the bottles used in an experiment: mix the material well and thereby produce a uniform pool.

3. Plant 3 cc. of this pool to each tube of encystment medium. Each tube contains 8-10 cc. of liquid medium which covers the agar slant.

4. Observe at intervals for maximum encystment—usually 55 to 60 hours.

5. Pipette off all liquid, leaving the sediment.

6. Wash the sediment three times with distilled water, centrifuging between washes.

7. Standardize each tube to five cc. by adding distilled water.

8. Store in ice box 24 hours and then make hemacytometer count of cysts.

In order to evaluate the efficiency of the several methods, as used in our hands, ten consecutive weekly comparisons of cyst production on the different media were made. Mother cultures were grown in both Boeck and Dobohlav's medium (Ringer's-Egg-Serum) and on Cleveland and Collier's Difco medium with 2% agar, since some workers have used one and some the other.

Table I shows the results of this comparative test in our hands. Minimum and maximum and average counts of thousands of cysts per cc. are recorded for the ten tests. In order to determine the total number of cysts procured in one test tube, the recorded figure should be multiplied by five.

It will be observed that : 1) For the most part trophozoites grown on liver infusion agar without starch produced greater quantities of cysts when added to the various encystment media containing starch than the trophozoites grown on Ringer's-Egg-Serum Media without starch. Cleveland's liver infusion agar medium therefore appears to be the better medium on which to grow the mother cultures without starch prior to encystment.

<sup>3</sup> In recent conversation, Chang states that he is now using with improved results a liver-infusion agar base over which he places the buffered liver-infusion liquid.

2) Of the five media tested, the Buffered Liver Infusion Agar produced the greatest number of cysts with the greatest consistency.

3) Ordinary unbuffered Liver Infusion Agar produced the fewest cysts.

TABLE I  
*Comparison of encystment media*

	THOUSAND CYSTS PER CC. IN EACH CULTURE		
	Min.	Max.	Av.
Amoebae stock cultures in Boeck-Drbohlav's Ringer's-Egg-Serum without starch...			
Encystment media with starch			
Boeck-Drbohlav's Ringer's-Egg-Serum.....	5	44	18.2
Cleveland-Collier Difco-Entamoeba medium (2%-agar)....	2	34	12.6
Locke's Solution and Rice Starch—Army Medical School.....	4	24	8.6
Chang's Buffered Liver-Infusion Broth.....	5	63	27.1
Buffered Liver Infusion Agar...	10	99	50.0
Amoebae stock cultures in Cleveland-Collier Difco-Entamoeba Medium (2%-agar) without starch			
Encystment media with starch			
Boeck-Drbohlav's Ringer's-Egg-Serum.....	36	114	65.5
Cleveland-Collier Difco-Entamoeba Medium (2%-agar)....	4	32	13.8
Locke's Solution and Rice Starch—Army Medical School.....	10	66	36.9
Chang's Buffered Liver-Infusion Broth.....	13	113	64.0
Buffered Liver Infusion Agar...	71	366	209.3

### *Procedures*

In preliminary studies in the early part of this work, ten cc. of water, containing cysts were subjected to the chlorine or ozone tests with consistent results, but in the tests recorded in the graphs in this paper by preference one liter samples of water were used throughout. The liter of water containing the cysts for chlorination was placed in a 2000 cc. lipped flask. To this the chlorine was

added and the flask corked. At the time the experiment was concluded the necessary amounts of the mixture for pH and chlorine residual tests were poured over the lip from the flask, thus removing any untreated cysts that might have adhered to the upper rim of the tested material. The balance was immediately neutralized with sodium thiosulfate.

The ozone tests were made by placing the liter sample of material to be tested in a 1500 cc. lipped cylinder and the ozone was conducted to the bottom of the tube in air and diffused through the sample by means of an alundum extraction thimble of medium porosity. At the end of the time intervals, samples for pH and ozone residual were removed by pouring over the lip of the tube, and the balance was neutralized. In both tests the residual values represent oxidation residuals measured by orthotolidine and expressed on the customary chlorine basis.

Within one minute following the neutralization of the samples tested, 200 cc. was poured from the flask, 100 cc. into each of two 100 cc. centrifuge tubes, and centrifuged for 5 minutes at 3000 r.p.m. The supernatant, 99½ cc., was siphoned off each tube and the bottom ½ cc. from each tube which contained the cysts was pooled and planted into R. E. S. medium and incubated for 72 hours. Controls for each test consisted of 200 cc. of the test sample which had stood at the temperature in question for the longest time interval of the experiment, an amount of sodium thiosulfate identical with the amount used in the tests always being added to the control. Only experiments where controls demonstrated more than a minimum of 5 trophozoites per field using an 8 mm. objective and 5 × ocular were retained as suitable for the experiment. Negative culture tubes were checked again at 96 hours before being discarded as negative.

In the experiments summarized in the graphs, 5 or more tests were performed for each point represented and numerous determinations were likewise made at the time intervals below and above these points. During the course of the experiment with liter samples of water, 291 tests were performed. Each time interval plotted on the graphs represents the highest bactericidal and cysticidal time obtained at that particular pH level and time interval.

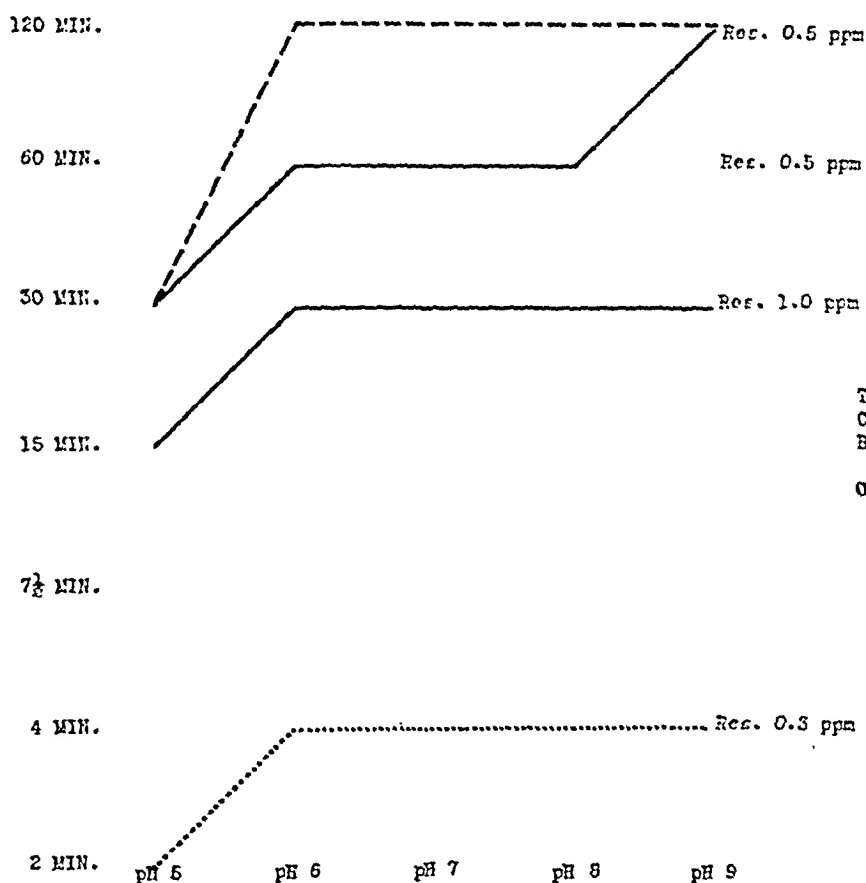
The N. R. S. strain of *E. histolytica* was used throughout and only cysts that were less than 10

days of age were selected for experiments. One hundred cysts per cc. were selected as standard. The bacterial count of 500,000 to 1,000,000 per cc. represented the bacteria in the amoebae cultures usually present in these dilutions. In the latter experiments of the series, following the standardization of the buffered liver infusion agar procedures, bacterial counts following the washings were found to be much lower than during the first part of the work and in some instances fresh bacterial culture had to be added to the sample to produce

results and since no critical comparative tests have been made at these low residuals, the highest residual employed is recorded.

The temperature selected for all experiments was 27°C. and the organic nitrogen ranged from 0.5 to 2.00 p.p.m. The time intervals were selected as being representative of geometric progression, and the pH determinations ranged from 5 to 9.

The actual dosages in p.p.m. are not recorded since they varied slightly in each experiment, probably being largely dependent upon the amount



GRAPH I. CYSTICIDAL ACTION OF CHLORINE AND OZONE

the count established in the earlier experiments. The bacteria recovered from our cultures were *Escherichia coli*, *Alkaligenes fecalis*, and a strain of gamma *Streptococcus*. *Escherichia coli* was the only organism recovered in the plate counts following the test.

Although the ozone residual is recorded as 0.3 p.p.m., this represents the highest residual used in these experiments. It fell below this point a few times, actually ranging between 0.1 p.p.m. and 0.3 p.p.m. No differences were observed in the

of organic nitrogen present. In general they ranged from two to three times the required residual.

#### DISCUSSION AND CONCLUSIONS

The following observations may be made from the graphs. The conditions under which the tests were made must be recalled in their interpretation (Graph I, cysticidal tests).

1. At pH ranges between 6 and 8 the cysticidal time of gaseous chlorine producing a residual of

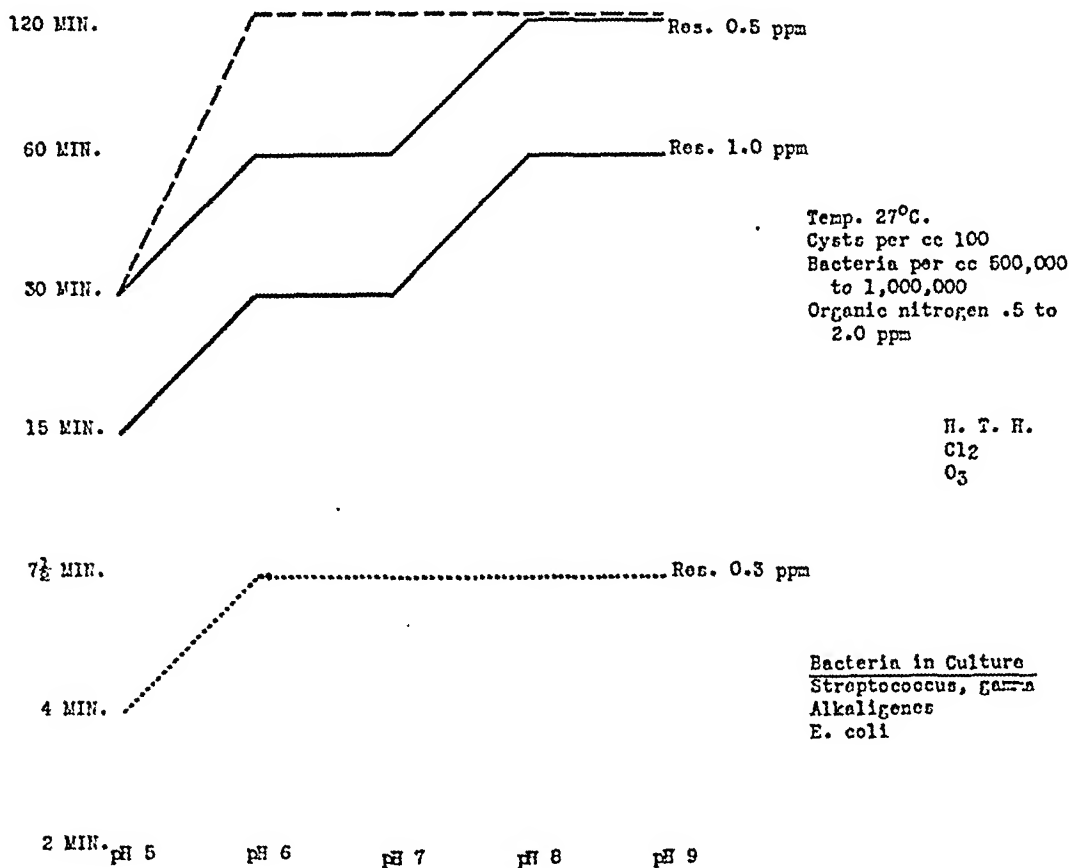
0.5 p.p.m. was sixty minutes while the cysticidal time of calcium hypochlorite (H. T. H.) was one time interval higher, or 120 minutes.

2. At pH of 5 the cysticidal time for both gaseous chlorine and H. T. H. becomes 30 minutes and at a pH of 9 the cysticidal time of the gaseous chlorine increases to the 120 minute level.

3. Gaseous chlorine producing a residual of 1 p.p.m. is cysticidal in 15 minutes at a pH of 5, and in 30 minutes at pH levels ranging from 6 to 9.

1,000,000 per cc. represents a heavy load in comparison with average bacterial counts obtained in water bacteriology. With lower counts, bactericidal time intervals would have been reduced throughout.

1. At pH ranges of 6 and 7 the bactericidal time of gaseous chlorine, producing a residual of 0.5 p.p.m., was 60 minutes while the bactericidal time of H. T. H. was one time interval higher or 120 minutes.



GRAPH II. BACTERICIDAL ACTION OF CHLORINE AND OZONE ON BACTERIA IN CULTURES OF *ENDAMOEBIA HISTOLYTICA*

4. Ozone producing a residual of 0.3 p.p.m. was cysticidal at 2 minutes at a pH of 5 and in 4 minutes at pH levels between 6 and 9.

The cysticidal action of chlorine on *E. histolytica* observed in this laboratory study, when compared with the same points in the studies of Stone (5), Chang and Fair (6) and Brady, Jones and Newton (7) in general shows similar results.

In reviewing the results recorded on Graph II, bactericidal tests, it should be emphasized that a bacterial count varying between 500,000 and

2. At pH levels of 5, 8 and 9 the bactericidal action of gaseous chlorine and H. T. H. was the same.

3. The bactericidal action of gaseous chlorine producing a residual of 1 p.p.m. was 1 time interval lower than the bactericidal action of gaseous chlorine producing a residual of 1 p.p.m.

4. The bactericidal time of ozone producing a residual of 0.3 p.p.m. was 4 minutes at a pH of 5 and 7½ minutes at pH levels ranging from 6 to 9.

In comparing the bactericidal load ranging be-

tween 500,000 and 1,000,000 per cc. with the cyst load of 100 cysts per cc. in the two graphs, it is seen that the bactericidal and cysticidal results do not vary appreciably from each other.

1. The results with H. T. H. and gaseous chlorine producing a residual of 0.05 p.p.m. were both 30 minutes at pH 5 and 120 minutes at pH 9. Bactericidal and cysticidal results were likewise identical at pH levels of 6 and 7 though they varied one time interval between the H. T. H. and gaseous chlorine. The only difference was at pH 8 where the bactericidal results were 1 time interval higher than the cysticidal results.

2. The bactericidal and cysticidal results with gaseous chlorine, producing a residual of 1 p.p.m., were identical at pH ranges of 5, 6, 7 but the bactericidal times were one interval higher at pH levels of 8 and 9.

3. At pH of 5, the bactericidal results with ozone producing a residual of 0.3 p.p.m. fall at 4 minutes, while the cysticidal time was 2 minutes. At pH levels varying from 6 to 9 the bactericidal time was 7½ minutes while the cysticidal time was 4 minutes.

4. In both bactericidal and cysticidal studies the ozone producing a residual of 0.3 p.p.m. was several times more effective than either H. T. H. producing a residual of 0.5 p.p.m. or of gaseous chlorine producing residuals either of 0.5 p.p.m. or of 1.0 p.p.m.

#### SUMMARY

A comparison of the cysticidal and bactericidal effects of chlorine and ozone is made at pH levels ranging from 5 to 9 in which the test solutions were buffered to hold at constant levels for the duration of the experiment. Cysts of *E. histolytica* and the bacteria occurring in the routine cultures of the same were tested. One hundred cysts per cc. were selected as a standard dosage for the experiments, the bacterial count accompanying this dosage ranging between 500,000 and 1,000,000 per cc. Chlorine residuals of 0.5 p.p.m. and 1.0 p.p.m. were compared with an ozone residual of 0.3 p.p.m. at time intervals ranging from 2 minutes to 240 minutes at a temperature of 27°C. The following generalizations may be made:

1. Gaseous chlorine producing a residual of 0.5 p.p.m. when compared with H. T. H. producing the same residual was in general more active.

2. The activity of chlorine tended to decrease at the higher pH levels studied, while the activity

of ozone was less at pH 6 than at pH 5, but was not reduced between pH 6 and pH 9.

3. No great differences were noted between the bactericidal and cysticidal effects of the loads tested. It must be noted that the bacterial load tested was unusually heavy, and if a bacterial load of 100 to 1000 per cc. were tested the bactericidal times would have been lower.

4. The bactericidal and cysticidal times required by ozone producing a residual of 0.3 p.p.m. were several times less than those required by chlorine producing residuals of either 0.5 p.p.m. or 1.0 p.p.m.

5. A buffered liver infusion agar base with a buffered serum mixture is described as an encystment medium for *E. histolytica*. The results of cyst production on this medium are compared with cyst production on Boeck-Drbohlav's Ringer's-Egg-Serum, Cleveland-Collier's Difco-Entamoeba Medium (2% agar), Locke's Solution and Rice Starch, and Chang's Buffered Liver-Infusion Broth. In our hands the buffered liver infusion agar was superior in cyst production to the other media tested.

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# AMEBIASIS OF THE UTERUS<sup>1</sup>

DAMASO DE RIVAS

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Amebiasis is the term commonly used to indicate an infection of the body by unicellular organisms known as amebas. These parasites of which several species have been described, commonly are found in the gastro-intestinal tract where they may give rise to pathological changes. Of special clinical importance is *Endamoeba histolytica* which commonly invades the large intestine and is the cause of amebic dysentery, also called tropical dysentery or intestinal amebiasis. This parasite may also directly invade the lower part of the ileum and appendix and cause appendicitis and by metastasis may be carried to the liver and lungs and give rise to amebic abscesses in these organs.

The case to be reported is that of a woman, M. B., aged 70 years, who was in a hospital for mental diseases as a senile case for about four years, she never was out of the country. The blood showed a rather marked degree of secondary anemia, pernicious in type. Before her death, October 27, 1942, she had intermittent attacks of diarrhea for about six weeks, the feces were not examined for parasites.

There was nothing of importance in the physical history of the case that would have had any definite bearing on the cause of death except that the physician in charge of the case, noticing a rather profuse vaginal discharge, on examination found the uterus enlarged, the cervix swollen, soft and ulcerated and cancer was suspected.

At the autopsy I did not find any pathologic changes of importance to account for the immediate cause of death beyond a generalized arteriosclerosis, senile changes of the internal organs and a moderate degree of hypostatic pneumonia. Next in importance was the examination of the uterus for the evidence of cancer.

Macroscopically the uterus was found moderately enlarged and soft. The cervix was swollen,

soft, eroded and ulcerated and showed several small hemorrhagic areas. Microscopical examination of the sections showed a subacute ulcerative inflammation, congestion, sero-cellular infiltration and granulation tissue, no evidence of a neoplastic growth was found. On further examination, however, quite unexpectedly and to my surprise, I detected several round and oval bodies in the submucosa below the ulcers and also into the deeper layers of the tissues. A more detailed study of the sections revealed these bodies to be the vegetative form of *Endamoeba histolytica* as shown in the accompanying illustrations.

## COMMENT

In reviewing the recent literature I find that Morse and Seaton, American Journal of Tropical Medicine, May 1943, describe the presence of amebas in the vaginal discharge of two cases in China, Herman and Berman, Journal of A. M. A., November 1942, claim to have detected amebas in the discharge of an ulcer of the penis and Shih, Wu and Lieu, Chinese M. J. February 1939, report a case of amebiasis of the penis, but the actual invasion of the tissues by the amebas in these cases is not stated by the authors.

## SUMMARY

The author, to the best of his knowledge, believes this to be the first case of amebiasis of the uterus to be reported in this country.

The invasion of the sex organs by amebas opens a new field of investigation as to the probable transmission of *Endamoeba histolytica* by sexual intercourse.

I desire to express my thanks to Dr. Charlotte Sweeney Helmar, for her assistance at the autopsy, Miss Eliza Gunn and Mrs. Virginia Rich, for the preparation of the tissue sections and to Dr. Otterbein Dressler and Mr. Joseph Poppel, for their assistance in the preparation of the illustrations.

<sup>1</sup> Read at the Thirty-Ninth Annual Meeting of the American Society of Tropical Medicine, Cincinnati, Ohio, November 16-18, 1943.



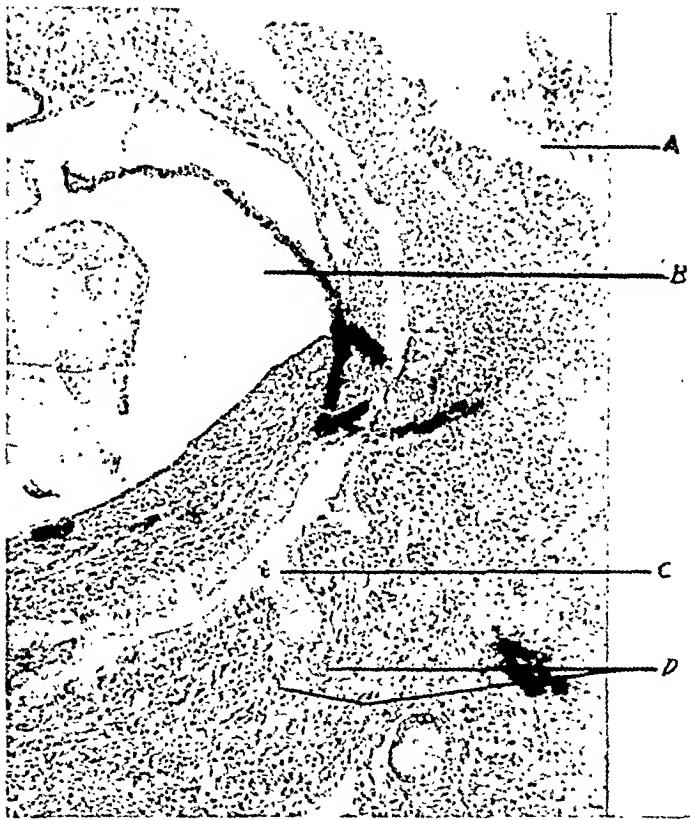


FIG. 1. AMEBIASIS OF THE UTERUS

Cervix showing A, ulceration. B, cyst. C and D, *Endameba histolytica* in recess of mucosa and submucosa respectively.

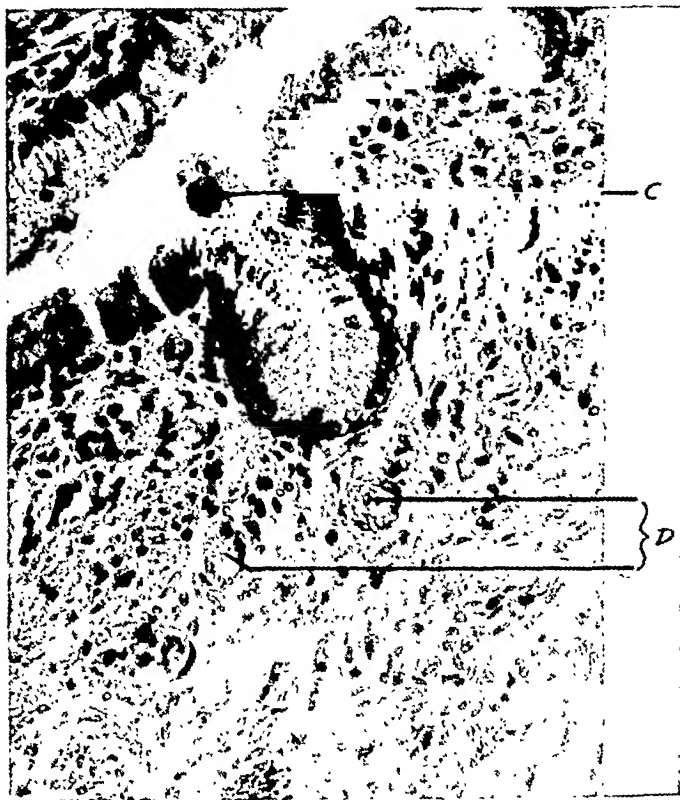


FIG. 2. AMEBIASIS OF THE UTERUS

Same as Fig. 1, magnified showing *Endameba histolytica* in recess of mucosa, C and D in submucosa

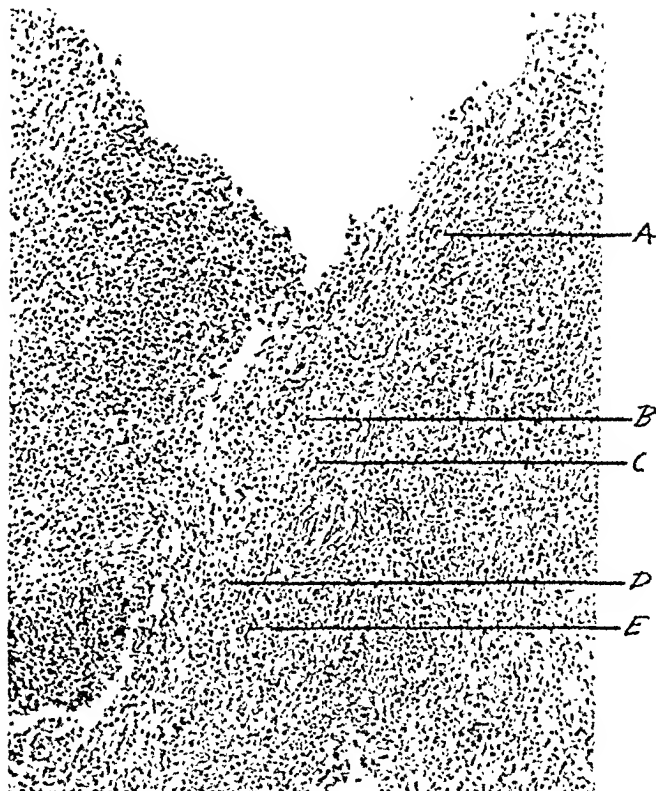


FIG. 3. AMEBIASIS OF THE UTERUS

Cervix showing ulceration and ser-cellular exudate and several *Endameba histolytica* in submucosa: A, B, C, D and E.

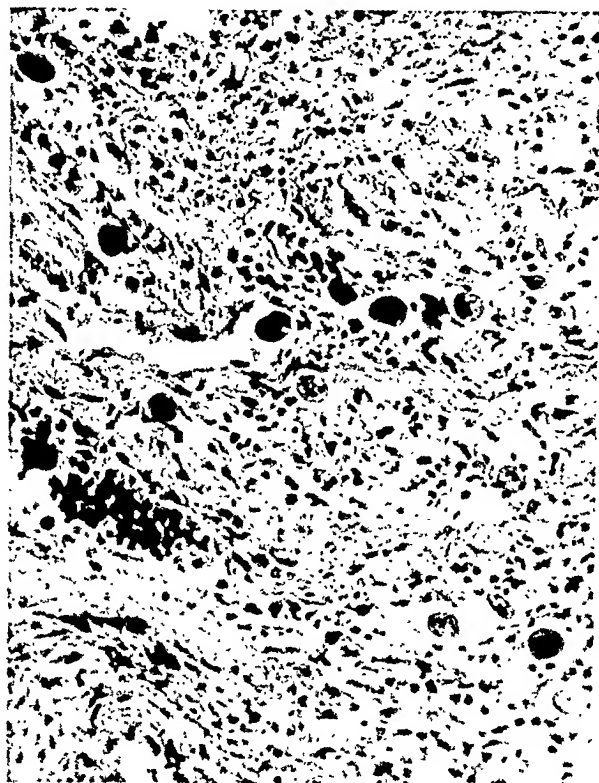


FIG. 4. AMEBIASIS OF THE UTERUS

Same as Fig. 3, magnified showing *Endameba histolytica* in the submucosa and deeper part of the tissue



# THE INFLUENCE OF CHOLESTEROL AND CERTAIN VITAMINS ON THE GROWTH OF *ENDAMOEBIA HISTOLYTICA* WITH A SINGLE SPECIES OF BACTERIA<sup>1</sup>

CHARLES W. REES, JOHN BOZICEVICH, LUCY V. REARDON, AND FLOYD S. DAFT

*From the Divisions of Zoology and Chemotherapy, National Institute of Health, United States Public Health Service, Bethesda, Maryland*

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During the past several years the nutritional requirements of *Endamoeba histolytica* have been investigated in this laboratory with the view of improving the cultures which are used in the production of antigens. Our ultimate objective is the development of a medium which will supply all the growth factors necessary for the cultivation of the amoeba in high concentrations without bacteria or other agents that interfere with the specificity of antigens. Up to the present time, (1, 2, 3, 4, 5) the amoebae have been freed from the bacteria of the stool and cultivated with a single species that has been designated as organism *t*. In our modification of the L.E.S. medium in Erlenmeyer flasks average yields of 1,000,000 amoebae per flask have been reported. From these amoebae antigens of high potency and specificity have been processed and used in the complement fixation test for amoebiasis. Experiments on amoeba-organism *t* cultures have shown that much hydrogen is produced by the bacteria in the L.E.S. medium thus facilitating an anaerobic environment which, according to Snyder and Meleney (8), is a growth prerequisite for the amoebae; also that reducing substances diffusing from the solid to the liquid phase may function in the nutrition of the organisms. A hitherto unpublished observation that amoebae failed to grow on a once used base that was replenished with fresh overlay has strengthened the evidence that amoebae are nourished by soluble products of egg. Our failure in a number of other unpublished experiments to demonstrate any effect of vitamins

or other substances on the growth of amoebae was probably due to their initial occurrence in whole egg. A discovery that media made either from egg white or egg yolk are deficient in nutritive factors has provided the basis for the work presented in this paper.

## PREPARATION OF MEDIA

Medium was prepared for stock tube cultures as previously reported (6) and in addition in 250 ml. Florence flasks which were used instead of Erlenmeyer flasks (4) because the shape of the former provides for more gradual diffusion of reducing substances and for less surface area between air and liquid. The solid phase contained 50 ml. of a particular coagulum and 3 gm. of crushed egg shell per flask; the liquid phase contained 200 ml. of Locke's solution (NaCl, 8 gm.; KCl, 0.2 gm.; CaCl<sub>2</sub>, 0.2 gm.; KH<sub>2</sub>PO<sub>4</sub>, 0.3 gm.; dextrose 2.5 gm. and distilled water 1,000 ml.) supplemented with 0.03 gm. of rice flour and with or without enrichment substances as listed in table 1. Whole egg coagulum was prepared as in previous work (4) with 4 eggs to 50 ml. of Locke's solution, egg white coagulum with 6 whites to 50 ml., and yolk coagulum with 6 yolks to 25 ml. An albumin coagulum, A<sub>2</sub>F<sub>3</sub>C (table 1) was prepared from a 10 per cent commercial product that we call "glassy" albumin. In two media the yolk base was enriched with 2 gm. of powdered and "glassy" albumin, respectively. The wholly liquid medium, IF<sub>3</sub>C (table 1), was prepared with 50 ml. of an infusate of coagulated egg white and 200 ml. of Locke's solution per flask.

Cholesterol (Merck's cholesterin), used in 13 experiments, was suspended in distilled water, sterilized in the autoclave, emulsified by shaking with glass beads, and added to the flasks in the amount of 0.1 mg. per ml. of the liquid phase of the medium. Cholesterol sodium sulfate, used in the remaining experiments, was dissolved in distilled water, sterilized in the autoclave, and added

<sup>1</sup> Read at the Thirty-Ninth Annual Meeting of the American Society of Tropical Medicine, Cincinnati, Ohio, November 16-18, 1943.

We wish to thank Senior Surgeon Lloyd D. Felton of the Division of Infectious Diseases, Principal Statistician William M. Gafafer of the Division of Industrial Hygiene, and Passed Assistant Surgeon (R) Roy Hertz of the Division of Chemotherapy for valuable suggestions.

TABLE 1

*Comparative yields of Endamoeba histolytica from various media in Florence flask cultures*

COMPOSITION OF MEDIUM	DESIGNATION	NUMBER OF TESTS	HARVEST OF AMOEBAE IN TENS OF THOUSANDS PER FLASK*		
			Minimum	Maximum	Average
Whole egg.....	WE	20	24	274	153.0
Egg white.....	A	20	1	45	20.5
Egg white enriched with the following:					
Cholesterol.....	AC	4	11	55	25.0
Lecithin.....	AL	1			12.0
Cholesterol and lecithin.....	ACL	2	10	23	16.5
Vitamins, amino acids, and purine bases (Solutions I, II, and III).....	AF <sub>1</sub>	3	10	24	19.0
Vitamins and purine bases (Solutions I and III).....	AF <sub>2</sub>	2	28	84	56.0
Vitamins, amino acids, and purine bases (Solutions II and III).....	AF <sub>3</sub>	2	16	52	34.0
Vitamins and amino acids (Solutions I and II).....	AF <sub>4</sub>	1			28.0
Vitamins (Solution I).....	AF <sub>5</sub>	2	28	54	41.0
Vitamins, amino acids, purine bases, and cholesterol (Solutions I, II, and III).....	AF <sub>1</sub> C	3	99	171	128.0
As above plus lecithin.....	AF <sub>1</sub> CL	6	53	169	96.0
Vitamins, purine bases and cholesterol (Solutions I and III).....	AF <sub>2</sub> C	2	77	197	137.0
As above plus lecithin.....	AF <sub>2</sub> CL	1			110.0
Vitamins, amino acids, purine bases, and cholesterol (Solutions II and III).....	AF <sub>3</sub> C	2	52	57	54.5
As above plus lecithin.....	AF <sub>3</sub> CL	1			64.0
Vitamins, amino acids, and cholesterol (Solutions I and II).....	AF <sub>4</sub> C	3	81	133	109.0
As above plus lecithin.....	AF <sub>4</sub> CL	2	25	50	37.5
Vitamins and cholesterol (Solution I).....	AF <sub>5</sub> C	9	90	160	125.0
Vitamins and cholesterol (Solution IV).....	AF <sub>6</sub> C	4	65	115	93.0
Vitamins and cholesterol (Solution V).....	AF <sub>7</sub> C	1			17.0
Vitamins and cholesterol (Solution VI).....	AF <sub>8</sub> C	1			19.0
Commercial "glassy" albumin enriched with vitamins and cholesterol (Solution I).....	A <sub>2</sub> F <sub>5</sub> C	1			9.0
Egg yolk.....	Y	12	7	51	23.0
Egg yolk enriched with the following:					
Vitamins, amino acids, purine bases, and cholesterol (Solution I, II, and III).....	YF <sub>1</sub> C	1			14.0
Vitamins and cholesterol (Solution I).....	YF <sub>2</sub> C	4	2	21	11.0
Ovalbumin.....	YO <sub>1</sub>	1			7.0
Ovomucin.....	YO <sub>2</sub>	1			56.0
Ovomucoid.....	YO <sub>3</sub>	1			43.0
Ovalbumin, ovomucin, and ovomucoid.....	YO <sub>1</sub> O <sub>2</sub> O <sub>3</sub>	2	72	73	72.5
Commercial powdered albumin.....	YA <sub>1</sub>	1			18.0
Commercial "glassy" albumin.....	YA <sub>2</sub>	1			53.0
Difco Bacto-Peptone.....	YP	1			18.0
Wholly liquid infusate of egg albumin, vitamins, and cholesterol (Solution I).....	IF <sub>5</sub> C	1			70.0

\* Based on the combined counts of 2 to 3 workers on the pooled harvests from 5 flasks.

*Components of solutions:*

Solution I. Thiamine hydrochloride, riboflavin, niacin, pyridoxine hydrochloride, calcium pantothenate, choline, inositol, and p-aminobenzoic acid.

Solution II. Biotin, B-alanine, and casein hydrolysate.

Solution III. Adenine, guanine, thymine, uracil, and salt solution B.

Solution IV. Thiamine hydrochloride, riboflavin, and niacin.

Solution V. Pyridoxine hydrochloride, and calcium pantothenate.

Solution VI. Choline, inositol, and p-aminobenzoic acid.

in the amount of 1 microgram per ml. of the liquid phase. Soybean lecithin was used and prepared in the same manner as the cholesterol and added in the amount of 0.5 mg. per ml. of the liquid phase.

The vitamins, amino acids, and purine bases, formulae  $F_1$  to  $F_8$  inclusive, were prepared in 6 solutions, and were added to the egg white and yolk media in the combinations shown in table 1. Solution I was added in the amount of 0.5 ml. per flask and contributed 0.1, 0.2, 0.1, 0.1, 0.2, 2.5, 2.5, and 1.5 micrograms respectively of thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, niacin, calcium pantothenate, choline, inositol, and p-aminobenzoic acid per ml. of the overlay. Solution II was also added in the amount of 0.5 ml. per flask and contributed 0.00004, 0.25, and 50 micrograms respectively of biotin, B-alanine, and casein hydrolysate per ml. of the overlay. Solution III was added in the amount of 1 ml. per flask and contributed 1 microgram of adenine, guanine, thymine, and uracil and 0.001 ml. of salt solution  $B^2$  (7) per ml. of the overlay. Solution IV was added in the amount of 0.1 ml. per flask and contributed 0.1, 0.2, and 0.1 micrograms respectively of thiamine hydrochloride, riboflavin and niacin, per ml. of the overlay. Solution V was added in the amount of 0.1 ml. per flask and contributed 0.1 and 0.2 micrograms respectively of pyridoxine hydrochloride and calcium pantothenate per ml. of the overlay. Solution VI was also added in the amount of 0.1 ml. per flask and contributed 2.5, 2.5, and 1.5 micrograms respectively of choline, inositol and p-aminobenzoic acid per ml. of the overlay.

Ovalbumin, ovomucin, and ovomucoid were made up in 5, 2.5, and 2.5 per cent solutions respectively and, after sterilization in the autoclave, 2 ml. of each solution were added to each flask. Difco Bacto-Peptone was added in the amount of 10 per cent of the yolk base.

#### EXPERIMENTAL PROCEDURE

Seventy-two hour cultures of *E. histolytica*-organism *t* in test tubes formed the stock material from which all flasks were inoculated. The inoculum consisted of the sediment of 2 tubes per flask and from the latter the amoebae were harvested after 72 hours of incubation, the pooled yield from 5 flasks forming the standard for determining the

effectiveness of each experimental medium. The liquid from each lot of flasks was reduced by centrifugation to a 10 ml. concentrate and transferred to a 250 ml. beaker to secure samples for counting. The samples were obtained in a Pasteur pipette while the beaker was being gently shaken. The counting was done with a Neubauer hemocytometer as for leucocytes. Each harvest was counted by 2 and in most cases by 3 workers, each of whom counted 2 samples. The figures reported are the means of the combined counts. The limits of variability and other statistical constants will be discussed in another publication.

Although several well known methods of enumerating bacteria have been tried, none has been found suitable for routine counting of organism *t* in our cultures; research is needed to devise a satisfactory method of counting these organisms or of measuring their metabolic activity.

#### RESULTS OF EXPERIMENTS

A summary of the most pertinent data is contained in table 1 and shows that there were striking differences between the yields from whole egg, egg white, and egg yolk media, with the last two showing scanty growth. However, certain of the enrichment formulae added to the egg white medium enhanced the growth of the amoebae, producing yields almost as good as those from whole egg medium. Vitamins of the B group had no effect unless supplemented with cholesterol; conversely, cholesterol alone had no effect. Additional experiments are needed to determine the effect of amino acids of casein hydrolysate and the 4 purine bases.

In five experiments enrichment of the yolk medium with the vitamin-cholesterol ingredients had no demonstrable effect. With few exceptions, our efforts to determine effective supplemental factors for yolk medium have thus far been unsuccessful.

Two brands of commercial albumin did not support the growth of amoebae either when coagulated as a base and supplemented with vitamins and cholesterol or added to yolk medium. Further work is needed on ovalbumin, ovomucin, and ovomucoid. Our supply of these substances has thus far been inadequate. In addition to the Difco Bacto-Peptone experiment noted in table 1, several other experiments with this product or with Difco Proteose-Peptone indicated that neither had any demonstrable effect on the growth of amoebae.

<sup>2</sup> Salt solution B contains  $MgSO_4 \cdot 7H_2O$ , 10 gm.;  $NaCl$ , 0.5 gm.;  $FeSO_4 \cdot 7H_2O$ , 0.5 gm.;  $MnSO_4 \cdot 4H_2O$ , 0.5 gm.; distilled water, 250 ml.

The yield from whole egg medium in Florence flasks was higher than that previously reported (4) from Erlenmeyer flask cultures. The differences between the minimum, maximum, and average yields of the whole egg medium were very pronounced: in 4 of the 20 experiments the yield was below 1,000,000 per flask while in 6 it was above 2,000,000. This variability emphasizes the need for further research to determine the causative factors.

#### DISCUSSION

The need for methods of enumerating amoebae more accurate than those currently in use was stressed by Paulson (9) and was apparent also in the present investigation. We have followed this author in adapting the well established leucocyte counting technique to our experimental conditions. Although in need of further refinement, this technique has enabled us to measure differences that could otherwise not have been determined between the yields of the formulae presented in table 1.

Data not reported in table 1 appear to indicate that differences between lots of eggs may have contributed to the variability shown between the amoeba harvests from whole egg medium. For example, the harvests were scanty in some lots of media made from stale eggs and from fertile eggs incubated up to 8 days.

The advantages of cultivating *E. histolytica* with a single bacterial symbiont were emphasized in previous work (4) and also by the investigations of Snyder and Meleney (8) who found that a medium supporting good growth of amoebae with mixed bacteria would not support growth with a single species. With a single species of bacteria these workers found serum indispensable for growth of amoebae whereas in our *E. histolytica*-organism *t* cultures this thermolabile ingredient was not required. Snyder and Meleney cultivated *E. histolytica* under anaerobic conditions with mixed bacteria in a cysteine-peptone-cholesterol medium and reported that peptone was indispensable. However, with a single species of bacteria in anaerobic culture the effectiveness of peptone was not demonstrated. In our *E. histolytica*-organism *t* cultures the addition of peptone failed to promote growth. Differences between the bacterial species appear, therefore, to have been important factors governing the effectiveness of serum, peptone, and other ingredients of the medium. Thus the type of flora is of primary importance in studies on the cultural requirements of this amoeba.

In agreement with Snyder and Meleney, we have found that cholesterol is essential for the growth of *E. histolytica*. Cailleau (10) believed that cholesterol acts in the nature of a vitamin for the nutritional requirements of *Eutrichomaslix colubrorum* and certain species of *Trichomonas*. The present studies indicate that certain vitamins of the B complex group are essential for the growth of *E. histolytica*, and further that certain as yet undetermined components of egg white are likewise essential in meeting the nutritional requirements of this organism when grown with a single species of bacteria.

#### SUMMARY

In Florence flask cultures of the L.E.S. medium the average yield of *Endamoeba histolytica*, based on 20 experiments, was 1,530,000 per flask but was scanty in media made either from egg white or egg yolk.

The addition to egg white medium of cholesterol and 8 vitamins of the B complex group stimulated growth of *E. histolytica* to a point well over 1,000,000 amoebae per flask but was without effect on yolk medium.

Cholesterol alone or the vitamins alone in egg white medium had no effect on growth of *E. histolytica*.

The counts of amoebae obtained from medium enriched with the amino acids of casein hydrolysate and with the 4 purine bases were slightly higher than those from an unenriched medium.

Two commercial products of dehydrated egg albumin did not support growth of *E. histolytica*.

Ovalbumin, ovomucin and ovomucoid when added in combination to yolk medium showed some stimulating effect on the growth of *E. histolytica*.

In one instance an infusate of coagulated egg white plus the vitamins and cholesterol produced a fair crop of amoebae.

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# THE INCIDENCE AND SIGNIFICANCE OF TRICHOMONAS VAGINALIS INFESTATION IN THE MALE

LOUIS G. FEO<sup>1</sup>

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## I. INTRODUCTION

Accurate statistics are not available as to the frequency of *Trichomonas vaginalis* in men, but it is the opinion of many that the incidence of infestation is higher than the literature would lead one to believe.

Surveys have been made to determine this incidence in large groups of men though the methods used are open to some criticism. Of 32,000 examinations of prostatic secretions, reported from the Mayo Clinic (1), the presence of *Trichomonas vaginalis* was revealed 16 times. Dastidar (2) examined about 1,000 specimens of urine and found trichomonads in the urine of 3 males and 1 female. *Trichomonas vaginalis* was reported to be the etiologic factor in 5 cases of the 500 non-gonorrheal discharges analyzed by Lehman (3), while Knight and Shelanski gave 10.4 as the percentage of *Trichomonas vaginalis* found in 500 cases of male urethral discharges (4). An attempt to determine the probable incidence of *Trichomonas vaginalis* among the male population was made by Liston and Lees (5). Investigating a consecutive series of 400 men attending a venereal disease clinic, they found that approximately 4 per cent of the males suffered from *Trichomonas vaginalis* infestation.

The opportunity to examine consecutively a large series of men who had the provisional diagnosis of urethral discharge was afforded the author while assigned to the Genito-Urinary Section of the Station Hospital at Fort George G. Meade, Maryland. The survey upon which the present study is based was undertaken to determine to what extent *Trichomonas vaginalis* might be found in men.

### *Material and method*

The men studied were admissions to the genito-urinary wards, following the routine physical inspection at the Recruit Reception Center. The admissions studied extended over seven months (September, 1942 to March, 1943). All inductees found to have a urethral discharge or suspicious

of having one are admitted for further study. The discharges, therefore, varied from the profuse, purulent, gonorrheal type to the clear drops of urine, resulting from a distended bladder. This group of examinees is considered representative of the male population of the states of Pennsylvania, Maryland, and Virginia, and the District of Columbia.

Of the 1,000 men examined, 926 provided urethral specimens. The others were diagnosed as cases of balanitis or presented a chancre or chancroid lesion, and were not included in this study. Of the 926 men investigated, 735 were negroes and 191 were white. Their ages varied from eighteen to forty-three years, with the mean falling between twenty and thirty years.

The specimens were collected and examined in the following manner. The men were examined upon arising in the morning before attending the latrine. The genitalia were clearly exposed. The urethra was "stripped" carefully and the resulting discharge collected on a cotton swab previously moistened with normal saline. This was then mixed in 2-3 cc. of physiological salt solution. The organisms were sought by the microscopic examination of the moist films. Most of the specimens were studied immediately upon collection, the others within two hours. Cultures were not prepared.

## II. RESULTS

### *Incidence*

Table 1 gives the numbers and percentages of incidence for the different etiologic agents for the examination of the 926 men. The figures are based on one examination per person, with the exception of 24 trichomonad-positive individuals. These revealed smears with numerous organisms and were repeatedly examined over a period of one month. These repeated examinations showed a persistent infestation, without apparent diminution in numbers of organisms. Further reference will be made to this particular group.

Of the 735 negroes examined, 407 (55.37 per

<sup>1</sup> Captain, Medical Corps, Army of the United States.

cent) were positive for the gonococcus and 121 (16.46 per cent) showed *Trichomonas vaginalis*. Only 18 (2.45 per cent) of the total of 735 men harbored both the gonococcus and *Trichomonas vaginalis*. It was noted that *Trichomonas vaginalis* rarely was found in association with the thick, purulent, gonorrheal discharge. One hundred and eighty-nine others (25.71 per cent) showed neither organisms, and are grouped under the term of non-specific urethritis. When the trichomonad-positive cases were grouped with the non-specific ones, the percentage of cases of so-called non-specific urethritis revealing the *Trichomonas*

TABLE 1

Summary of data on the examination of the urethral discharges of 926 men

EXAMINEES	ETIOLOGY				TOTAL PERSONS
	<i>Neisseria gonorrhoeae</i>	<i>Trichomonas vaginalis</i>	Non-specific	<i>Trichomonas vaginalis</i> and <i>Neisseria gonorrhoeae</i>	
Negro men					
Number. ....	407	121	189	18	735
Per cent. ....	55.37	16.46	25.71	2.45	
White men					
Number. ....	110	23	57	1	191
Per cent. ....	57.59	12.04	29.84	0.52	
Totals					
Number. ....	517	144	246	19	926
Per cent. ....	55.83	15.55	26.56	2.05	

*vaginalis* was surprisingly high. For the negro group it was 39 per cent.

The number of white men examined was 191, and 110 (57.59 per cent) were positive for the gonococcus. Twenty-three (12.04 per cent) were infested with *Trichomonas vaginalis*, and one individual showed the presence of both the gonococcus and the flagellate. The incidence of *Trichomonas vaginalis* found in cases of non-specific urethritis in the white group was 28.7 per cent.

Of the 926 men reported here, 246 (26.5 per cent) were classed as non-specific urethritis. When the 144 positive *Trichomonas* cases were added to these, then the percentage incidence of non-specific urethritis cases which might be attributable to *Trichomonas vaginalis* was 36.9 per cent.

### Symptoms and signs

On questioning the trichomonad-positive group, there was little in the information supplied to indicate present or past symptoms related to the presence of the flagellates. Of the 24 men with persistently positive smears, 2 had sought medical aid previous to their induction into the Army. Both were conscious of a slight urethral discharge, usually upon arising in the morning. With the appearance of the discharge, there was a slight urethral irritation, described by the patients as a "burning" on urination. One had been conscious of this, intermittently, over a period of 18 years.

The urethral discharge of the trichomonad-positive group was slight in amount, varying from a watery, flecked with white, type to one that was greyish-yellow in color. All were thin in consistency. In general, the microscopic examination revealed few epithelial cells, few to moderate numbers of leucocytes and bacteria, with few motile trichomonads. Smears of the 24 persistently positive men showed numerous trichomonads plus bacteria, and the picture was similar to that seen in cases of *Trichomonas vaginalis* vaginitis.

### III. DISCUSSION

With the exception of the paper by Liston and Lees (4) there are no figures on the incidence of *Trichomonas vaginalis* in the male population. In the present study (926 men) there is a total of 144 cases harboring *Trichomonas vaginalis* (table 1). This is a percentage incidence of 15.5. Separating this positive group into white and negro men, the percentage incidence is 12 and 16.5 respectively. These figures are higher than those reported by the above English investigators. They undertook to estimate the extent to which *Trichomonas* infection may be found among men who are affected with urethral symptoms. Since it is generally known that the male may harbor the organism without symptoms, the data presented in table 1 are significant of a more accurate determination of the prevalence of *Trichomonas vaginalis* infestation in men.

Liston and Lees (5) reported that approximately 16 per cent of males suffering from non-gonorrheal urethritis were cases of *Trichomonas vaginalis* infestation. Grimm (6) reported finding the flagellate 5 times in a series of 25 cases of non-specific urethritis, and Nitschke (7) 5 positives in 40 cases of non-specific urethritis. In the present study the incidence is 36.9 per cent.

The male infested with *Trichomonas vaginalis* usually presents no symptoms. Often the only complaint is a slight watery discharge. This discharge is easily expressed from the anterior urethra where the trichomonads are located in the numerous glands of the urethral mucosa. This is in contrast to the female suffering from the same infection. The male urethral surface is small compared to the vaginal area, and is continually being flushed by the urine, allowing but slight accumulation of discharge. Although the incidence of infestation in men is high, they suffer such slight reaction that they are unaware of the disease until another organism invades the urethra, setting up a mixed infection. Then the discharge is more profuse and becomes purulent in character.

Among the 144 *Trichomonas*-positive cases there were 24 which repeatedly revealed numerous organisms, over a period of observation of one month. There were 8 white men and 16 negroes in this group. The discharge in this group also was slight, but usually of a dirty white or muco-purulent type. The gentle "stripping" of the urethra expressed a drop or two of discharge, which showed numerous motile trichomonads and bacteria. The stained smears showed few epithelial cells, and moderate numbers of pus cells. Bacterial cultures revealed *Staphylococcus albus*, diphtheroids and gamma streptococci. It is to be noted that many of these smears, gram-stained, are similar to vaginal smears from cases of *Trichomonas vaginalis* vaginitis, as to number of trichomonads and associated bacteria, especially the small gram-negative diplococci so frequently found in such cases.

The problem of *Trichomonas vaginalis* infestation in the male is important because of symptomatic trichomoniasis among the female population. The lack of symptoms in men and the reported low incidence of infestation has retarded to a large extent the removal of these organisms from women. Only after subjecting the female to repeated treatments, with poor results as to final elimination of the infection, has the attention of the Medical profession been directed to the male as a source of re-infection.

The high incidence of infestation reported in this survey (15.5 per cent) emphasizes the fact that the male is not only responsible for the re-infection of women, but is the principle agent of transmission. The ease with which *Trichomonas vaginalis* is implanted into negative vaginas has already been

established (8, 9). The present study also shows that many stained smears of Trichomonad-positive urethral discharge are similar to those obtained in cases of *Trichomonas vaginalis* vaginitis. These two factors facilitate the transfer of the infection from the male to the female during coitus.

It would appear, therefore, that the cause of a persistent infestation in the woman should be sought for in her consort and there also eliminated.

#### IV. SUMMARY

Inductees with a provisional diagnosis of urethral discharge were examined for the presence of *Trichomonas vaginalis*. Urethral specimens were provided by 926 men, of whom 735 were negroes and 191 were white. The method of demonstrating the organisms was the microscopical examination of moist films.

This study revealed 144 men positive for *Trichomonas vaginalis*, a percentage incidence of 15.5. Separating this group into white and negro men, the percentage incidence was 12 and 16.5 respectively.

Of the 926 men examined, 246 (26.5 per cent) were classed as non-specific urethritis. The incidence of *Trichomonas vaginalis* found in cases of non-specific urethritis was 28.7 per cent in the white group. For the negro group it was 39 per cent. The percentage incidence of non-specific urethritis cases which may be attributable to *Trichomonas vaginalis* was 36.9 per cent.

The entire group of *Trichomonas vaginalis*-positive men was relatively free from all symptoms. A discharge may be noted which is characteristically small in amount, thin in consistency, and of a dirty, white color. Microscopically, this discharge showed few epithelial cells, and a moderate number of pus cells and trichomonads. Some of the stained smears were similar to vaginal ones from cases of *Trichomonas vaginalis* vaginitis, as to number of trichomonads and types of bacteria.

The male is the important transmitter of *Trichomonas vaginalis* infestation, while the female eventually becomes a reservoir of infection.

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# INTRADERMAL REACTIONS FOLLOWING THE USE OF DIROFILARIA IMMITIS ANTIGEN IN PERSONS INFECTED WITH ONCHOCERCA VOLVULUS<sup>1</sup>

WILLARD H. WRIGHT AND JOHN R. MURDOCK<sup>2</sup>

*Pan American Sanitary Bureau, Washington, D. C.*

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During the summer of 1943 the writers were detailed to organize under the supervision of Doctor Hugh S. Cumming, Director, Pan American Sanitary Bureau, a program in the research and control of onchocerciasis to be carried out in cooperation with the Republics of Mexico and Guatemala. One of the phases of this program dealt with the epidemiology of the disease in these two countries and in this connection it seemed desirable to consider the problem of ascertaining the number of individuals who might be carriers of the disease and yet show no evidence of external nodules.

Van den Berghe (1, 2) demonstrated for the first time the presence of adult female *Onchocerca volvulus* in full reproductive activity outside of nodules and showed that of 1,704 individuals examined at Uele, Belgian Congo, 66.3 per cent had dermal microfilariae although only 61.2 per cent showed external nodules. The excess of those with dermal larvae he believed to represent individuals who harbored in the deeper connective tissues adult gravid female worms which could not be detected even with the most careful palpation.

In any successful control campaign, it would be necessary to detect such individuals and to take such steps as might be possible to eliminate them as reservoirs of infection. While skin biopsies constitute a method of diagnosis in these cases, a simpler test which could be carried out with less labor and equipment would be of more practical application in the inaccessible zones of onchocerciasis in Mexico and Guatemala.

## REVIEW OF LITERATURE

Hoffmann and Vargas (3) reported that they carried out intradermal tests in infected and non-

infected individuals, using 0.1 to 0.2 cc. of an antigen prepared by Gutiérrez (4) from *O. volvulus*. In infected persons, they noted a papule formation which reached its maximum in 2 hours and which was accompanied in some cases by pruritus and erythema. However, the injection of the material in non-infected individuals also resulted in the formation of a wheal and in some cases the wheal reached a diameter of 2 cm. The above-mentioned authors did not state the dilution of the antigen or the number of cases tested.

Fairley (5) recorded the testing of a case of *O. volvulus* with a *Dirofilaria immitis* antigen (presumably in a concentration of 1 per cent) and the development of a wheal 2.8 x 2.6 cm. in diameter with pseudopodia and a surrounding zone of erythema. Four hours later the arm was reddened, swollen, and painful, and the axillary gland became tender.

Rodhain and Dubois (6) conducted intradermal tests with *O. volvulus* antigen on 3 patients, of whom one was also infected with *Lea loa*. Two of the patients, including the one with the double infection, reacted positively while the third was negative. These authors tested 3 additional *O. volvulus* cases with *D. immitis* antigen which they obtained from Fairley and presumably used in the same concentration as that employed by the latter. Positive reactions were obtained in 2 cases, including one in which the nodule had been removed one year and 7 months previously. In the third case, the reaction was regarded as partially positive; in this case the *Onchocerca* cyst had been removed approximately 13 months prior to the test.

With reference to the tests by Fairley and by Rodhain and Dubois, Van Hoof (7) has stated, as follows:

"It has been easy to repeat these experiments made by the authors named with *D. immitis* extract by substituting for this worm *O. volvulus*. Though at the laboratory at Leopoldville these skin tests have given

<sup>1</sup> Read at the Thirty-Ninth Annual Meeting of the American Society of Tropical Medicine, Cincinnati, Ohio, November 16-18, 1943.

<sup>2</sup> Detailed from the United States Public Health Service.

uniformly positive results it has to be remembered that with its lack of specificity the method, when applied to natives who may be heavily and variously parasitized, has no longer any significance."

While theoretically *O. volutus* antigen should give more specific results in skin tests in onchocerciasis patients, *D. immitis* material is easier to obtain in quantity and would be of more practical use in large scale testing. Van Hoof was apparently the first investigator to seriously consider the lack of specificity of the intradermal test in individuals infected with parasites other than *O. volutus*. However, it appeared to us that it might be possible to screen out these non-specific reactions from other parasites by the employment of a suitable dilution of the antigen. During our stay at Huixtla, Chiapas, Mexico, opportunity was afforded to conduct intradermal tests with *D. immitis* antigen on a number of patients infected with *O. volutus*. We are greatly indebted to Doctor Luis Figueroa, Jefe, Centro Medico y Investigaciones de Onchocercosis, for his courtesy in providing facilities for the work and for his interest and cooperation in this and other studies at Huixtla.

#### MATERIALS AND METHODS

The antigens employed in these studies were prepared by Senior Zoologist John Bozicevich of the National Institute of Health. Adult *D. immitis* were removed from the heart of dogs sacrificed at Norfolk, Va. and were frozen in dry ice and transported in a vacuum jar to the Institute at Bethesda, Md. The worms were thawed, washed several times in physiologic saline solution to remove host protein and were desiccated in the Flösdorf-Mudd apparatus. The material was extracted with physiologic saline solution in the refrigerator for a period of 24 hours, centrifuged, and the supernatant fluid pipetted off to be used as antigen. This was sterilized by heating at 56°C. for one hour for 3 consecutive days after which suitable cultural tests were made for sterility. Phenol in an amount sufficient to provide an 0.3 per cent solution was added as a preservative. Dilutions were made on a dry weight basis. In all tests, injections were made with a similar dilution of canine serum in order to rule out positive reactions which may have been due to sensitivity to any host protein which might be present in the worm material. A further control test was made with physiologic saline solution.

Prior to the intradermal injections, each patient was tested by the scratch method with a 1:100 dilution of all antigens, in order to avoid the possibility of intense local reactions and anaphylactic shock in markedly sensitive individuals. Two patients gave such a marked reaction to the scratch test that it was deemed inadvisable to apply the intradermal test.

The intradermal tests were carried out by the injection into the skin of the forearm of approximately 0.1 cc. of antigenic material. The reactions were of the immediate type and usually reached their height in 15 to 20 minutes. A reaction was considered positive when the diameter of the antigen wheal exceeded that of the physiologic saline control wheal by 3 mm. or more, a standard which has been employed in previous studies carried out by the staff of the Division of Zoology, National Institute of Health (8, 9). The patients were observed for 48 hours or longer but in no case was a delayed reaction encountered.

#### RESULTS OF TESTS

The patients on whom the intradermal tests were carried out had suffered from onchocerciasis for periods varying between 1 and 20 years. They were either of pure Indian or mestizo blood. Seventeen of the patients had no demonstrable nodules at the time of the test but had had external cysts removed within a few months of the date of the test; three of the patients had cysts at the time of the tests. Of the 20 individuals tested, 19 showed microfilariae on skin biopsies while one was negative.

No information was available on the Wassermann or Kahn reactions in the individuals tested, although it is probable that some of them were syphilitic. Nearly all of them had a past history of malaria. At the time of the tests, the 20 patients showed various parasitic infections divided as follows: *Trichuris trichiura* in 13, hookworms in 2, hookworms and mal de pinta in 1, *Strongyloides stercoralis* and malaria in 1, *Ascaris lumbricoides* and malaria in 1, *Trichuris* and hookworms in 1, and *Ascaris*, hookworms and malaria in 1.

All of the 20 onchocerciasis patients tested with a 1:2000 dilution of the antigen reacted positively. The smallest wheal encountered in the group was 6 mm. and the largest 22 mm. in diameter. In 5 individuals pseudopodia extended outward from the wheal, the longest of these pseudopodia being 7 mm. In many patients, the wheal was sur-

rounded by a zone of erythema but in view of the fact that all patients were dark skinned, it was difficult to define the limits of the erythematous zone. Consequently, the erythema did not enter into the evaluation of the reaction. There was no correlation between the extent of the reaction and the duration of infection or the total number of cysts which the individuals had harbored.

Ten of the eleven patients reacted to a 1:4000 dilution of the antigen. The patient negative with this dilution gave a well marked reaction with the 1:2000 dilution. Incidentally, this patient had been infected for 20 years, a longer time than any other individual in the series, and had lost count of the number of cysts which he had had over this period of time. The patient was totally blind due to the long continued invasion of the structures of the eye by the microfilariae of the parasite.

Two of the onchocerciasis patients reacted to the canine serum control, one to a dilution of 1:2000 and the other to a dilution of 1:4000. However, both individuals gave much stronger reactions to the *D. immitis* antigen.

In order to rule out the possibility of cross reactions in the presence of parasites other than *O. volutus*, we tested 20 other individuals who served as controls. One of the controls was not known to harbor any parasite but the other 19 persons were infected, as follows: *Trichuris trichiura* in 3, hookworm in 8, *Ascaris lumbricoides* in 1, *Strongyloides stercoralis* in 1, *Enterobius vermicularis* in 1, *Dientamoeba fragilis* in 1, *Trichuris* and hookworm in 1, *Trichuris*, hookworm and *Ascaris* in 1, *Trichuris*, hookworm and *Wuchereria bancrofti* in 1, and *Endamoeba histolytica* and malaria in 1.

All 20 of the controls were tested with the 1:2000 dilution of the antigen and 11 gave positive reactions. Nineteen were tested with the 1:4000 dilution of the material and 7 gave positive reactions. Four of the controls were tested at Huixtla and 16 at Washington, D. C. Of those tested at the former place, one had lived all her life in an endemic zone of onchocerciasis, while another had visited various endemic zones off and on since 1929, although was not known to have acquired an infection. Both of these individuals reacted positively to the 1:2000 dilution of the antigen. The other two controls tested at Huixtla were the authors, both of whom reacted negatively, although both reacted positively to a 1:2000 dilution of the canine serum control.

The 16 other control individuals tested at Wash-

ington, D. C. had never been near endemic zones of onchocerciasis and had never been exposed to infection. They all harbored intestinal helminths. Nine of the 16 gave positive reactions to the 1:2000 dilution of the *D. immitis* antigen while 7 reacted positively to the 1:4000 dilution. Of the control patients other than the authors, 3 gave positive reactions to the canine serum control in a dilution of 1:2000; 2 of these also reacted to the 1:2000 *D. immitis* antigen. There was no correlation between the species of helminth parasite harbored by the controls and the reaction with the antigen. However, the 2 controls who harbored only protozoa failed to react to the *D. immitis* antigen.

#### DISCUSSION

The results obtained in the present series of tests emphasize the need for considerable care in evaluating intradermal tests for the diagnosis of helminth infections. In spite of the fact that all of 20 onchocerciasis patients gave positive reactions to a 1:2000 dilution of antigen prepared from *D. immitis* and 10 of 11 patients showed similar reactions to a 1:4000 dilution, the subsequent use of the material on persons harboring helminth parasites other than *O. volutus* resulted in a certain number of positive responses. Since all of the onchocerciasis patients were infected with one or more helminth parasites in addition to *O. volutus*, it was not possible to determine what percentage of the positive reactions to the antigen was due to a filarid reacting factor and what percentage represented responses to a helminth group reacting factor. Additional tests with more dilute solutions of the *D. immitis* antigen must be carried out before it can be determined whether this material is of any value for the diagnosis of onchocerciasis. The present writers were unable to proceed further in the matter because of the serious illness of one of them at Huixtla, a circumstance which necessitated suspension of the work.

Evidence indicates that protein material derived from the host of *D. immitis* would probably not be present in the antigen in sufficient quantities to provoke false positive reactions. In this connection, 3 of our controls reacted to the canine serum antigen but not to the *D. immitis* antigen. In the onchocerciasis patients and the controls reacting to the *D. immitis* antigen, the wheals were much larger and the response more striking than was the case with the canine serum material.

Reactions in the control patients to both types



of antigen are not believed to be related to allergic states in the individuals involved since none of them had a history of allergy. Furthermore, Bozicevich and Hutter (10) have shown in connection with the use of *D. immitis* antigen that most allergic individuals fail to react to this material in dilutions over 1:4000.

#### SUMMARY AND CONCLUSIONS

Intradermal tests with antigen prepared from the dog heartworm, *Dirofilaria immitis*, gave positive reactions in all of 20 individuals infected with *Onchocerca volvulus* when used in a dilution of 1:2000 and positive reactions in 10 of 11 such patients when employed in a dilution of 1:4000. In addition to *O. volvulus*, all of the patients were infected with one or more other helminth parasites.

Similar tests were carried out on 20 control individuals, 18 of whom had never been exposed to onchocerciasis. Two of the control persons who had lived or worked intermittently in endemic zones gave a positive reaction. The other 18 individuals harbored intestinal parasites and 16 of them had intestinal helminths. The two with intestinal protozoa reacted negatively to the intradermal test with *D. immitis* antigen in both of the dilutions employed. With a 1:2000 dilution, 9 of the 16 individuals with intestinal helminths reacted positively, while 7 gave a similar reaction with the 1:4000 dilution of the antigen.

Control tests were carried out with canine serum antigen in the same dilutions as the *D. immitis* antigen. Two of the onchocerciasis patients reacted positively to the injection of the serum antigen, while 5 of the controls reacted similarly. However, the distribution and nature of these reactions indicated that the small amount of host protein which might be contained in the *D. immitis* antigen would not be likely to produce false positive reactions.

Results of the present study indicate that at least part of the positive reactions in the onchocerciasis patients were non-specific in character

and due to a helminth group reacting factor in the antigen. Additional work may demonstrate the feasibility of using antigen dilutions capable of detecting cases of onchocerciasis without producing false positive reactions in the presence of other helminths.

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# INTRADERMAL AND SEROLOGICAL TESTS WITH *DIROFILARIA IMMITIS* ANTIGEN IN CASES OF HUMAN FILARIASIS

JOHN BOZICEVICH<sup>1</sup> AND A. M. HUTTER<sup>2</sup>

From the Division of Zoology, National Institute of Health, United States Public Health Service, and the United States Naval Hospital, Bethesda, Maryland

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Filariasis apparently due to *Wuchereria bancrofti* has proved to be an important health problem in troops operating in certain endemic areas, and the question of early diagnosis has been of considerable concern. Any method which could be used to differentiate the symptoms of streptococcal lymphangitis, epidermophyton lymphadenitis, funiculitis, and traumatic orchitis from those due to *Wuchereria bancrofti* would be of value in this connection.

Several investigators have already shown that in the filarids there is a common group reacting factor which is capable of eliciting an intradermal response in persons harboring filarial infections and that antigens prepared from these filarids can also be used in serological tests. Taliaferro and Hoffman (1) used a 1:200 dilution of a saline extract prepared from the dog heartworm, *Dirofilaria immitis*, as an antigen for cutaneous tests for the diagnosis of filariasis. Fairley (2, 3) employed 1:200 and 1:1,000 dilutions of saline extracts for intradermal testing. The above investigators found that the majority of the suspected cases of filariasis gave positive intradermal and complement fixation reactions.

Lloyd and Chandra (4) used for complement fixation tests an alcoholic extract antigen and one derived from the acetone-insoluble lipoids of *D. immitis*. Twenty-three positive reactions resulted in tests on 89 filariasis cases. Fifteen additional individuals harboring helminths other than *W. bancrofti* were tested and positive reactions obtained in 3 persons infected with *Dracunculus medinensis*. The acetone-insoluble lipoidal fraction was thought to give better results than the alcoholic antigen.

More recently, Dickson, Huntington, and Eichold (5) employed antigen derived from *D. immitis* in intradermal tests on 137 Navy personnel with symptoms of filariasis and obtained

positive immediate and delayed reactions in 114, or 83.1 per cent, of these individuals. Interpretation of these results is difficult because of the fact that these authors gave no information concerning the strength and intradermal dosage of the antigen.

Acton and Rao (6) failed to obtain positive intradermal results with antigens prepared from the adult guinea worm and from embryos of *Wuchereria bancrofti*, while hydrocele fluid was not specific. However, they had some degree of success with *D. immitis* antigen prepared according to Fairley's method.

Finally, Mohr and Lippelt (7) prepared an antigen from *Contortospiculum rhacae* from the South American "ostrich" and obtained positive complement fixation results on persons infected with *W. bancrofti*.

Most of the above-mentioned authors conducted tests with only one dilution of the antigen and no attempt was made to perform titration tests with the view of evaluating factors other than filariasis which might have influenced the reactions. For this reason, it was thought that more fundamental work should be conducted on the problem.

## PREPARATION OF *DIROFILARIA IMMITIS* ANTIGEN

Adult *D. immitis* were obtained from dogs sacrificed at the dog pound at Norfolk, Va.<sup>3</sup> The heart was removed and the right ventricle opened with sterile precautions. The adult worms were washed in several changes of sterile saline and were then retained in the last saline solution until a sufficient number had been collected for a final washing in distilled water. The worms were removed immediately from the distilled water to prevent bursting of the females. In order to prevent bacterial growth due to any possible contamination during the process of removal from the heart, the worms were placed in sterile test

<sup>1</sup> Senior Zoologist, U. S. Public Health Service.

<sup>2</sup> Lieutenant Commander, M.C., U. S. Navy (R).

<sup>3</sup> The authors wish to express their gratitude to Mr. D. A. Robertson, State Game Warden, Norfolk, Virginia, for the privilege of obtaining the adult *D. immitis*.

tubes which were corked and transported in a thermos jar filled with dry ice. A hypodermic needle was inserted through the thermos jar cork to allow the carbon dioxide gas to escape.

On arrival at the laboratory on the following morning the worms were in a frozen condition. In spite of the prolonged freezing, some revived after thawing. They were removed from the test tube, thoroughly cut up with fine scissors, and then ground in the moist state in an agate mortar. The material was then placed in a desiccator containing phosphoric anhydride and the air evacuated to about 5 mm. of mercury. After several days it was again ground in the agate mortar.

The antigen was prepared on a dry weight basis with an initial dilution of 1:100 in physiologic saline solution. The extraction was allowed to proceed for 24 hours in the refrigerator after which the material was subjected to slow freezing. It was alternately frozen and thawed twice. It was then placed in a water bath at 56°C. for 4 hours with occasional shaking. After removal from the water bath, the material was centrifuged at 15,000 R.P.M. for 15 minutes. Fractional sterilization at 56°C. for 1 hour was resorted to until representative samples showed no bacterial growth either under aerobic or anaerobic conditions. Phenol was added to give a final concentration of 0.3 per cent.

To exclude false positive reactions which might be caused by host protein being carried over with the worms, blood serum from a noninfected laboratory reared dog was prepared in a manner identical with that of the *D. immitis* antigen and employed as one of the foreign protein control antigens. Also, to further exclude the possibility of previous sensitization to foreign protein, trichina antigen prepared according to Bozicevich (8) was employed as a second protein control. Actually the use of this material as a control offered some disadvantages since it is possible that some of the subjects may have harbored subclinical infections with *Trichinella spiralis*, in which event it is also possible that they may have given a specific reaction to the material. However, we have had extensive experience with this antigen and had knowledge of the most suitable titer for intradermal use. The third control consisted of physiological saline solution containing 0.3 per cent phenol.

#### INTRADERMAL TESTS

The quantity of antigen injected intradermally was 0.01 cc., an amount sufficient to raise the smal-

lest possible wheal. The reactions were read in 15 minutes,  $\frac{1}{2}$  hour, 1 hour, and 24 hours after the injection. A reaction was considered positive when the diameter of the antigen wheal exceeded by 3 mm. or more that of the control wheals.

Two types of reaction were encountered. The immediate type of reaction appeared within a few minutes after the injection of the *D. immitis* antigen and was at its maximum by 15 minutes. This reaction consisted of a wheal, with or without pseudopodia, surrounded by a zone of erythema and usually accompanied by an intense pruritus. The delayed type of reaction consisted of a large confluent swelling with some erythema. These delayed reactions were elicited only occasionally and in each case the individual had previously shown an immediate reaction. In some cases the delayed response did not appear for several days.

It was obvious from the studies of previous investigators that the optimal dilution for intradermal testing had not been determined. Consequently, we began our intradermal titration of the antigen at a 1:1,000 dilution on individuals who had not been in endemic areas of filariasis. It was found that the 1:1,000 dilution of the antigen gave false positive reactions in approximately 30 per cent of non-exposed individuals and that the same concentration of the various protein control antigens likewise provoked reactions in some individuals. However, in a 1:2,000 dilution the antigen was more specific and most positive reactions in non-infected individuals could be screened out by this dilution.

Since the worms from which the antigen was derived could conceivably contain a sufficient amount of blood serum from the host to provoke a false positive reaction in individuals sensitive to such protein, a number of allergic individuals were tested as controls. Our results in these cases indicated that the 1:2,000 dilution provoked a considerable number of false positive reactions. However, when the antigen was further diluted to 1:4,000, most of those false positive reactions were obviated. Additional tests were carried out on allergic individuals with a 1:8,000 dilution of the *D. immitis* and the various control antigens. The results of these tests are shown in table 1. It will be noted that only one patient gave a positive intradermal response. However, this individual also gave a reaction to the dog protein control antigen.

Since a rise in temperature is encountered in some cases of filariasis, attempts were made to

evaluate the influence of pyrexia on reactions to the intradermal test. For this purpose, we selected for test a number of malaria patients. The tests were carried out with a 1:8,000 dilution of the *D. immitis* and the control antigens. The results are recorded in table 2. To the best of his knowledge none of these individuals had been in endemic areas of filariasis. One gave a positive reaction to the

gen in the same dilution as employed in the *D. immitis* antigen, although the response was not as marked as in the case of the test antigen. Two of the patients reacted to the trichina antigen. One patient reacted to all the materials, including the saline control; this individual was apparently sensitive to all of the antigenic substances as well as to the preservative.

TABLE 1

*Intradermal tests with a 1:8,000 dilution of Dirofilaria immitis and other antigens in allergic individuals*

PATIENT	DIAGNOSIS	DIROFILARIA IMMITIS ANTIGEN	DOG PROTEIN ANTIGEN	TRICHINELLA SPIRALIS ANTIGEN	SALINE CONTROL
S. D.....	asthma	0	1 x 2	0	0
F. D. G.....	asthma	0	0	1 x 2	0
C. K. M.....	asthma	0	0	0	0
J. F. P.....	asthma	0	0	0	0
J. A. H.....	asthma	1 x 2	0	0	0
M. J. P.....	asthma	3 x 4	3 x 4	1 x 2	0
	hay fever				
C. E. S.....	hay fever	0	0	0	0
G. V. C.....	hay fever	0	1 x 2	0	0
W. M. H.....	hay fever	0	0	0	0
W. S. F.....	asthma	0	0	1 x 2	0

Measurements in mm.

*D. immitis* antigen but this individual also reacted to the dog protein antigen to the same degree and should therefore be classed as negative. One other individual gave a reaction to the dog protein control but failed to react to the *D. immitis* antigen. One patient reacted positively to the *Trichinella spiralis* antigen but not to the remaining antigens.

Table 3 shows the results of intradermal tests on 25 individuals who had been exposed to infection with *W. bancrofti* in edemic areas abroad. Effort was made to isolate microfilariae from the peripheral circulation of these individuals by the examination of thick smears, by following the procedure described by Knott (9), and by xeno-diagnosis carried out by feeding *Aedes aegypti* mosquitoes on the patients between the hours of 9 p.m. and midnight and later examining the mosquitoes for microfilariae. In spite of prolonged search, no microfilariae were encountered. However, all of the patients showed clinical symptoms consistent with those of filariasis and the diagnosis was made on these grounds.

It will be noted from table 3 that all these individuals gave positive reactions to the 1:8,000 dilution of the *D. immitis* antigen. However, 6 showed a reaction to the dog protein control anti-

TABLE 2

*Intradermal tests with a 1:8,000 dilution of Dirofilaria immitis and other antigens in malaria patients*

PATIENT	DIRO- FILARIA IMMITIS ANTIGEN	DOG PROTEIN ANTIGEN	TRICH- NELLA SPIRALIS ANTIGEN	SALINE CONTROL
C. E. E.....	0	3 x 4	0	0
L. R. H.....	0	0	0	0
O. A. A.....	0	0	1 x 2	0
J. S.....	0	0	3 x 4	0
T. W. N.....	3 x 4	3 x 4	0	0
J. W. F.....	0	0	0	0

Measurements in mm.

#### PASSIVE TRANSFER STUDIES

Blood was taken from 3 filariasis cases who gave positive intradermal reactions to the 1:8,000 dilution of *D. immitis* antigen but no reactions to the control substances. Each donor supplied sufficient serum to inject 2 recipients. Five of the six recipients had never been out of the continental limits of the United States. The sixth, R. H. G., had been in Mexico and Honduras; none had ever been in the vicinity of Charleston, South Carolina.

Each recipient received 4 injections of 0.1 cc. of

TABLE 3

*Intradermal reactions in the filariasis patients with a 1:8,000 dilution of Dirofilaria immitis and other antigens*

PATIENT	DIRO-FILARIA IMMITIS ANTIGEN	DOG PROTEIN ANTIGEN	TRICHINELLA SPIRALIS ANTIGEN	SALINE CONTROL
R. L. C.....	7 x 9*	3 x 3	0	0
R. J. A.....	5 x 6	0	0	0
J. J. N.....	7 x 8*	4 x 5	3 x 3	3 x 3
J. L. M.....	5 x 7*	1 x 2	0	0
W. L. C.....	5 x 6	1 x 2	0	0
R. N. S.....	7 x 8	4 x 4	0	0
B. G. P.....	7 x 9*	1 x 2	0	0
J. P. S.....	5 x 6	0	0	0
W. R. S.....	3 x 4	0	1 x 2	0
V. S. P.....	5 x 6	2 x 4	0	0
E. B. L.....	7 x 9*	1 x 2	0	0
H. J. G.....	7 x 9*	0	0	0
L. H. B.....	5 x 7*	1 x 2	0	0
C. J. K.....	5 x 7*	0	3 x 4	0
F. O. G.....	5 x 7*	2 x 3	0	0
C. J. S.....	5 x 7*	0	0	0
P. R. T.....	7 x 9*	0	0	0
W. E. F.....	5 x 7*	1 x 2	0	0
W. G. G.....	3 x 4	0	0	0
D. D. N.....	3 x 4	0	0	0
R. F. T.....	3 x 4	0	0	0
J. R. B.....	7 x 9*	0	0	0
R. L. J.....	3 x 4	0	0	0
F. C. M.....	7 x 9*	0	0	0
D. L. B.....	5 x 7	3 x 4	0	0

Measurements in mm.

\* Pseudopodia.

serum intradermally in the right arm. The sites were separated from each other by a distance of about 5 cm. Twenty-four hours later each prepared site was injected intradermally with 0.01 cc. of the following materials. Site 1 received a 1:8,000 dilution of *D. immitis* antigen, site 2 a 1:8,000 dilution of dog protein, site 3 a 1:8,000 dilution of trichina antigen, and site 4 physiologic saline solution containing 0.3 per cent phenol. The left arm which had not received any serum was injected with the above materials at the same time to serve as a control.

As shown in table 4, the intradermal test with *D. immitis* antigen in the prepared sites of the recipients gave immediate positive reactions in 4 of 6 individuals. Furthermore, the erythema and pruritus were about as intense as in the donors and the other filariasis patients. In 2 of the recipients, no intradermal response was elicited. In the control areas of the left arm, the recipients failed to give a reaction to the *D. immitis* and *T. spiralis* antigens, and the saline control. However, one recipient gave a positive reaction to the dog protein antigen. This individual had been handling laboratory dogs for over a year.

#### COMPLEMENT FIXATION STUDIES

The following technique was employed in performing the complement fixation tests. The serum was used undiluted as well as in dilutions up to 1:16. The antigen was employed in dilutions of 1:100 to 1:1,600. Varying dilutions of both the serum and antigen in 0.1 cc. amounts were used in

TABLE 4

*Intradermal reactions with a 1:8,000 dilution of various antigens in recipients sensitized 24 hours previously with blood serum from filariasis patients*

PATIENT		SENSITIZED ARM				CONTROL ARM			
Donor	Recipient	<i>D. immitis</i> antigen	Dog protein antigen	<i>T. spiralis</i> antigen	Saline control	<i>D. immitis</i> antigen	Dog protein antigen	<i>T. spiralis</i> antigen	Saline control
W. L. C.....	O. K.	3 x 4	1 x 2	0	0	0	3 x 4	0	0
W. L. C.....	C. D. B.	3 x 4	0	1 x 2	0	0	0	1 x 2	0
J. R. B.....	R. C. C.	7 x 9*	3 x 4	0	0	0	0	0	0
J. R. B.....	C. S. C.	3 x 4	1 x 2	0	0	1 x 2	1 x 2	0	0
W. R. S.....	R. H. G.	1 x 2	0	0	0	0	1 x 2	0	0
W. R. S.....	S. H. M.	0	1 x 2	0	0	0	0	0	1 x 2

Measurements in mm.

\* Pseudopodia.

each test. To each tube were added 2 units of guinea pig complement contained in 0.8 cc. The complement had previously been titrated with the antigen used in the test. The tubes were incubated for 1 hour at 37°C. After removal from the water bath, the hemolytic system consisting of 0.5 cc. of a 2.5 per cent suspension of sheep cells and 2 units of amboceptor contained in 0.5 cc. was added to each tube. Before reading, the tubes were incubated for an additional hour in a water bath at 37°C. All necessary control tests were performed on the test serum, the negative serum, and the antigen. No positive complement fixation reactions were encountered in the patients in this series.

With the view that perhaps our saline antigen did not contain sufficient antigenic potency, an alcoholic antigen was prepared by extracting 1 gram of powdered *D. immitis* with 50 cc. of absolute alcohol for 24 hours at 37°C. The material was centrifuged in a manner similar to that employed in the preparation of the saline antigen after which 0.5 per cent cholesterol was added. The anti-complementary dose and hemolytic action were determined. Both the saline antigen and the alcoholic antigen were titrated against rabbit anti-*D. immitis* serum by the complement fixation technique described above. With the saline antigen diluted 1:1,600, strong positive complement fixation tests were obtained with serum diluted 1:16. However, negative complement fixation tests were obtained with the alcoholic antigen in all dilutions of both antigen and serum.

#### TOXIC PROPERTY OF *D. IMMITIS* MATERIAL

With a view of immunizing laboratory animals, 12 rabbits were injected intraperitoneally with 100 milligram amounts of the residue which remained from the saline antigen; the material was suspended in 5 cc. of saline. One rabbit died 2 hours following the injection and 2 other rabbits died during the night. On necropsy there was no evidence of traumatism or bacterial infection. The remaining 9 rabbits were given a second injection of residue 3 weeks later and one animal died 22 hours after this injection. A week later, the 8 remaining rabbits were given a third injection of the material. One animal died 3 hours later and the second died 10 hours following this injection. The third animal died from undetermined causes 2 months after the last injection. The time interval between

injections could not be controlled as this was determined by the availability of our antigenic material.

Since only 5 of 12 animals placed on experiment remained alive at the end of 2 months following the third injection, the residue material evidently contained some toxic substance. Such a conclusion was further indicated by allergic manifestations elicited in a laboratory assistant who ground *D. immitis* powder on 2 occasions and suffered from marked periorbital edema.

#### DISCUSSION

The number of intradermal and serological tests in our study was limited because of our small supply of adult *D. immitis*. Nevertheless, our results indicate that the use of a 1:8,000 dilution of *D. immitis* antigen along with the recommended control materials is of value in arriving at a diagnosis of filariasis.

We are not in agreement with Taliaferro and Hoffman (1) or with Fairley (2, 3) in their belief that the use of intradermal antigens of the strength employed by them is of diagnostic aid in cases of filariasis. Although the 1:8,000 dilution of dog serum contains more foreign protein than would be carried over in the digestive tract of the adult *D. immitis*, past and present experience indicates that a host protein should be employed as a control. Furthermore, since an occasional individual will react to the preservative, it is advisable to employ in all control solutions the preservative used in the antigen proper.

Emphasis must be placed on the fact that the dilution of *D. immitis* antigen should be such as to provide positive reactions in filarial infections and not provide similar reactions in the presence of other parasites. Many previous workers have failed to observe this principle and have used materials which in many cases gave non-specific reactions. In our series of tests only 4 individuals had a history of nematode infections other than *W. bancrofti*. Two had harbored hookworms, one hookworms and *Trichuris*, and one *Trichuris*. These individuals had been given anthelmintic treatment and showed no evidence of infection at the time the present tests were conducted. A number of the patients had protozoan infections but apparently these did not influence the reactions.

It is of interest to note that the intradermal test

was followed by an exacerbation of symptoms of lymphangitis in 7 individuals and by pain in the scrotum and lymph glands, particularly the inguinal, in 8 other patients. This phenomenon may be connected in some manner with the presence of toxic materials such as were evidenced by the rabbit immunization results or with a particularly powerful antigenic factor present in the filarid group.

It is obvious from the local passive transfer studies that there is some skin sensitivity to *D. immitis*, since 4 of 6 individuals gave the Prausnitz-Küstner reaction. However, not all individuals can be used for local passive transfer studies as 2 of the recipients failed to react.

Fairley (2, 3) and others obtained positive complement fixation results in persons having filariasis and their work suggests that the method might be employed in diagnosis. However, our technique failed to give any positive reactions to this test. Fairley employed an alcoholic antigen for his tests while we used a saline antigen. However, our saline antigen contained more antigenic potency than did our alcoholic antigen when both antigens were used simultaneously against the serum of a rabbit which had been immunized to the residue from the saline antigen.

#### SUMMARY

All of 25 individuals suspected of having *Wuchereria bancrofti* infections gave positive intradermal reactions with a 1:8,000 dilution of an antigen prepared from *Dirofilaria immitis* while false positive reactions in non-infected and allergic persons were screened out by the use of this dilution. Only 4 of the 25 individuals had harbored helminth parasites other than *W. bancrofti* and sufficient evidence is therefore not available from our tests to indicate whether the above-mentioned dilution of the antigen would satisfactorily screen out false positive reactions which might be due to these other parasites.

In some cases the injection of the 1:8,000 dilution of the antigen was followed by exacerbations of the symptoms of lymphangitis together with pain in the scrotum and inguinal glands.

Negative complement fixation tests were ob-

tained in the above-mentioned cases with saline antigen ranging in dilutions from 1:100 to 1:1,600, although positive results were obtained in rabbits immunized with the residue from the antigen preparation. The alcoholic antigen appeared to be inferior to the saline material.

By testing 24 hours later with the *D. immitis* material, local passive hypersensitivity to *D. immitis* antigen was elicited in 4 of 6 recipients injected with the blood serum of filariasis patients.

Seven of 12 rabbits died while being immunized to the residue obtained from the antigen preparation, a fact which would seem to indicate that the adult worms contain some toxic substance.

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# REPORT ON THE PROGRAM FOR IMPROVING THE TEACHING OF TROPICAL MEDICINE IN THE MEDICAL CURRICULUM<sup>1</sup>

HENRY E. MELENEY

*From the Department of Preventive Medicine, College of Medicine, New York University*

It is a well known and regrettable fact that tropical and other parasitic diseases occupied a very small place in the curriculum of most of the medical schools in this country prior to the present war. Recognizing the urgent need for improvement, the Association of American Medical Colleges appointed a Committee on the Teaching of Tropical Medicine at its annual meeting in October 1941. The Committee consisted of Dr. Maxwell E. Lapham of Tulane University, Dr. Malcolm H. Soule of the University of Michigan and myself.<sup>2</sup> This Committee made a survey of the instruction being given in the medical schools (1, 2), which showed that 20 per cent gave no required instruction in parasitic diseases and 62 per cent gave no required instruction in tropical medicine. In addition, 29 per cent gave inadequate instruction in parasitic diseases and 30 per cent gave inadequate instruction in tropical medicine. The report also showed that 29 per cent of the schools had no teaching personnel with special training in parasitology and 50 per cent had no teaching personnel with special training in tropical medicine. The data suggested, however, that in a number of schools more adequate instruction could be given by the personnel already available.

Before the submission of the Committee's report to the Association of American Medical Colleges in October 1942, consultation with Brigadier General James S. Simmons and with Mr. Archie S. Woods of the John and Mary R. Markle Foundation indicated that the Army and the Markle Foundation were interested in providing opportunities for instructors from medical schools to obtain intensive instruction in tropical medicine. Accordingly the Committee recommended that the Association sponsor such a program, and the Association instructed the Committee to proceed with its development. The Surgeon General of

the Army offered to permit instructors to attend the eight weeks' course in tropical medicine at the Army Medical School, and Tulane University offered to give a special course in tropical medicine of the same length. The Markle Foundation made a grant of \$25,000 to the Association, later increased to \$35,000, to pay travel and maintenance expenses and tuition at Tulane University. Each medical school was offered the opportunity of sending one of its instructors to each of these courses. The first course started at each institution on January 2, 1943. Provision was made for thirty instructors to attend each course. Subsequently, other instructors have attended later courses at both schools. A total of 84 instructors from 60 medical schools have had the advantage of these courses up to the present time. Canadian as well as United States schools have been represented. Soon after this program was started, General Simmons and Mr. Woods suggested that it would be advisable for these and other instructors who had had training or were interested in tropical medicine to obtain a brief period of practical experience in the American tropics. The United Fruit Company, the Office of the Coordinator of Inter-American Affairs, the Pan American Sanitary Bureau, the Army and the Division of Medical Sciences of the National Research Council offered to cooperate in this program. In February 1943, a group of seven, consisting of General Simmons, Colonel Frank B. Wakeman, Brigadier General Leon A. Fox, Dr. Francis G. Blake, Mr. Woods, Dr. W. C. Davison and the Chairman of the Committee, made a short tour of Central America to investigate possible centers for instruction. The Markle Foundation appropriated an additional \$35,000 to the Association of American Medical Colleges to finance this program. Arrangements were made for groups of about eight instructors to spend three weeks at a United Fruit Company hospital and one week with a local field unit of the Office of the Coordinator of Inter-American Affairs in one of the Central American countries. Hospitals in Guatemala, Costa Rica, Honduras and Panama, and field units in Guate-

<sup>1</sup> Read at the Symposium on War and Post War Tropical Medicine at the meeting of the American Society of Tropical Medicine, Cincinnati, Ohio, November 17, 1943.

<sup>2</sup> Dr. Hiram W. Kostmayer of Tulane University replaced Dr. Lapham in October, 1942.



mala, Honduras, El Salvador and Costa Rica were made available. The first group of instructors went to Central America in June and subsequent groups have gone monthly since then. Through the month of December, 56 instructors from 42 medical schools in the United States and Canada will have had the benefit of this experience. Certain changes have been made from time to time in the program, the chief one being the reduction in time spent at the United Fruit Company hospitals and the use of the general hospitals in Guatemala City and San Jose, Costa Rica, where local physicians of high caliber have offered their services and where abundant clinical material adds greatly to the value of the experience. The local field units of the Office of the Coordinator of Inter-American Affairs have arranged tours on which tropical diseases can be seen in their endemic areas and on which programs of prevention can be demonstrated.

Both the Central American program and the attendance at the Army Medical School course will be continued in 1944 for the benefit of instructors who could not be released earlier from their teaching responsibilities.

Another recommendation made to the *Association of American Medical Colleges* by the Committee was that a Distributing Center for teaching material in tropical medicine be established. After a questionnaire to the medical schools had revealed a need for such a center, the Army Medical School took the responsibility of establishing it and the National Institute of Health offered to cooperate in furnishing specimens. The Markle Foundation made an appropriation of \$1000 to meet incidental expenses. The Center began operating in January 1943. During the first six months of operation, 55 institutions, agencies and individuals furnished specimens for distribution, and during the first ten months of operation, 43,659 specimens were distributed. Not only medical schools, but army training centers, army hospitals and graduate schools have received teaching specimens. In addition, instructions have been distributed for the local collection and preparation of specimens. A list of illustrations suitable for lantern slides and a list of motion pictures had been prepared.

In June, 1942, a Distributing Center for pathological specimens was established at the Army Medical Museum. It received an appropriation of \$5000 from the Markle Foundation through the American Foundation for Tropical Medicine and

is furnishing gross and microscopic pathological material to the medical schools.

At the suggestion of General Simmons, the Markle Foundation made a grant of \$40,000 to the Division of Medical Sciences of the National Research Council in the summer of 1942 for the purpose of enabling specialists in tropical diseases to give lectures and demonstrations at medical schools. The object of this program is to reach especially fourth year students, hospital interns and faculty members, and to stimulate an interest in tropical medicine. Lecturers from both this country and from Latin America have participated in this program. Most of the medical schools have had at least one lecturer and some have had four or five.

Another contribution of the Preventive Medicine Division of the Office of the Surgeon General of the Army, again supported by a grant of \$5000 from the Markle Foundation, has been the publication of information on the diseases and medical services of individual tropical countries. The publication of this material in book form has been arranged through the National Research Council and it will be distributed in the near future to instructors teaching tropical medicine, medical libraries and other interested individuals and agencies. In addition, a series of 14 maps showing the world distribution of the important tropical diseases has been prepared, a set in wall size has been furnished to each school and a set in notebook size has been distributed to each fourth year medical student in the United States. This should contribute to the teaching program of these institutions.

It is impossible in this short review to mention by name the many individuals who have cooperated to make the program a success. This has been done in the Committee's report given at the annual meeting of the Association of American Medical Colleges last month (3).

Reports from medical school instructors who have attended the courses at the Army Medical School and Tulane University and from those who have obtained practical experience in Central America, indicate that these programs have been very profitable and have stimulated an interest which has been transmitted to the medical schools in the form of personal enthusiasm and an increase in the time, effort and cooperation devoted to tropical medicine in the medical curriculum. Even schools which have not participated in this

program are known to have improved their instruction. This, however, is only a beginning. The Commonwealth Fund and the Rockefeller Foundation have already granted travel fellowships to a few instructors for longer periods of experience in Latin America. The Markle Foundation is also interested in offering longer fellowships to those who have shown particular promise or interest in their short periods of training. Now that momentum has been developed in providing minimum immediate needs for the war period, it is necessary to look forward to the post-war period when our interests in the health of the entire world will be an important element in stabilizing the peace. Tulane, Columbia and possibly one or two other universities will undoubtedly develop permanent graduate schools of tropical medicine equal to those anywhere in the world, but the developments must be on a much broader basis. The contacts of instructors from many schools with institutions in Latin America have created a mutual interest which must be nurtured. This can be done not only by individual travel fellowships, but much more effectively by the development of official

relationships between universities in this country and in Latin America, by which an exchange of faculty members and students on a basis of equality would increase knowledge, stimulate research and contribute to friendly international relations. The American Society of Tropical Medicine, in cooperation with the Association of American Medical Colleges, may well make this a part of its future program for advancing tropical medicine throughout the world.

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# FINANCIAL SUPPORT OF TROPICAL MEDICINE<sup>1</sup>

ALFRED R. CRAWFORD

*Secretary, American Foundation for Tropical Medicine, Inc.  
Assistant to the President, Long Island College of Medicine*

The purpose of this presentation is three-fold:

1) to report on the activities of the American Foundation for Tropical Medicine during the past year;

2) to comment on the Foundation's position in relation to other sources of financial support for teaching and research in the field of tropical medicine and parasitology;

3) to consider some of the possibilities for further development of the Foundation's activities.

It may be useful to preface this report with a brief review of the Foundation's background. It is familiar to some of you, but others may find it helpful.

The American Foundation for Tropical Medicine was established in 1935 in Washington, D. C., by the American Academy of Tropical Medicine. The Academy had been organized the previous year. The purpose of the Foundation was to provide a medium for contributions from philanthropically-minded individuals and from corporations with interests in tropical regions. These contributions would be applied, in the words of an Academy report dated January 23, 1935, to the financial support of "institutions and groups . . . engaged in programs of research and applied work in this field."<sup>2</sup> Dr. Earl B. McKinley was the first executive director of the Foundation.

The report goes on to state that "it is believed that by a coordinated program, funds may be brought together for furthering work in tropical medicine in the various institutions interested in this field. Separately most institutions are finding it difficult to finance adequately the various needs for this type of work. Working together under a representative and competent organization, the full force of our needs may be better emphasized to prospective donors."<sup>3</sup>

<sup>1</sup> Report for presentation at meeting of American Society of Tropical Medicine, Cincinnati, Ohio, November 17.

<sup>2</sup> McKinley, E. B. "The Development of Tropical Medicine in the United States." Report of the Secretary, American Academy of Tropical Medicine, 1935.

<sup>3</sup> Ibid.

The tragic death of Dr. McKinley cut short the ambitious plans for the Foundation which were then contemplated. Re-established in 1940 and incorporated under the laws of the State of New York with Dr., now Lieutenant Colonel, Thomas T. Mackie as executive director, the Foundation became a substantial factor in financing the work of the graduate department of tropical medicine at Tulane University. Its support of the Tulane project in 1940-41-42 amounted to about \$36,000.

When Dr. Mackie entered the Army last year to become executive officer of the division of military and tropical medicine at the Army Medical School, he was succeeded as executive director by Dr. J. A. Curran.

The upsurge in interest in tropical medicine which coincided with this country's entry into the war had finally crystallized by the end of 1942 and was bringing a number of corporations which for long had been potential Foundation donors to the point of action. This provided the setting for the enlargement of the Foundation's sphere of activity which has characterized the current year.

A program calling for the collection and allocation of \$100,000 during 1943 was adopted at the annual meeting last January.

Through the efforts of the executive committee and several of the directors, results for the year may be reported as follows:

1) Twenty-three corporations have made gifts and pledges amounting to \$78,100.

2) Thirteen direct grants, all recommended for approval by the Medical Committee, have been made to 11 institutions of medicine, to the Army Medical Museum and to the Journal of Parasitology.

3) Eight of the institutional grants have been for the purpose of strengthening and extending the teaching of tropical medicine and parasitology on the undergraduate and postgraduate level, one for purposes of research and two for both teaching and research.

4) The Foundation, in cooperation with the Harvard University School of Medicine's Department of Comparative Pathology and Tropical

Medicine, is administering a fundamental investigation of African sleeping sickness in Liberia, West Africa, which includes the continuation of a program of treatment instituted in 1937 by the Firestone Plantations Company.

Besides Tulane, Harvard, the Army Medical Museum and the Journal of Parasitology, schools of medicine which have received grants this year have been Cornell, Duke, Manitoba, Nebraska, New York University, Pennsylvania, Stanford, Texas, Tufts and Yale.

The purposes of the various grants were as follows:

*Army Medical Museum:*

For payment of technicians and clerical assistance in the collection and distribution to medical schools of specimens illustrating the pathology of tropical diseases. This project was financed by a special grant from the Markle Foundation.

*Cornell University Medical College:*

Services of a full time instructor and research man in tropical diseases.

*Duke University School of Medicine:*

To establish identification and distribution center for pathogenic fungi and to maintain a registry for autopsy and biopsy material from cases of fungus disease. Grant to pay full-time mycologic and pathologic technician and to buy animals and supplies.

*Journal of Parasitology:*

To supplement publication funds to permit an enlarged type page, thereby increasing amount of material published.

*University of Manitoba Faculty of Medicine:*

Travelling fellowship for professor of parasitology and tropical diseases for special study at Johns Hopkins University.

*University of Nebraska College of Medicine:*

For one full-time technical assistant to assist teaching in student laboratories and for staff research in amebiasis and leprosy.

*New York University College of Medicine:*

Salary aid for full-time instructor in tropical medicine for second, third and fourth year students.

*University of Pennsylvania Medical School:*

Salary aid to finance expansion of teaching in parasitology and tropical diseases.

*Stanford University School of Medicine:*

Salary aid for instructor in tropical medicine who will also supervise preparation of new teaching material.

*University of Texas School of Medicine:*

To finance investigation of tick-borne rickettsial diseases on the Gulf Coast of Texas.

*Tufts College Medical School:*

To employ clinical teaching fellow in tropical medicine at Boston City Hospital.

*Tulane University School of Medicine:*

Budgetary needs of Department of Tropical Medicine.

*Yale University School of Medicine:*

To expand tropical medicine teaching at Yale by supplementing salaries of teaching and laboratory staff.

The first of a series of six-month progress reports have come in from beneficiaries. They reflect satisfactory progress and much useful work being accomplished.

Preliminary work on the research phase of the African sleeping sickness project has been accomplished at Harvard. The field treatment phase in Liberia has commenced and after the first of the year research activities will be transferred to Liberia. The project is established on a one year basis but may be extended to two years. With the cooperation of the Liberian government, which has been assured, there is good hope of further progress in the treatment and control of African sleeping sickness.

Such, in brief, is the story of the Foundation's activities in the current year to date.

## II

It seems pertinent to consider briefly the Foundation's position in relation to other agencies providing support to research, teaching and applied work in the field of tropical medicine and parasitology.

At least four sources of funds to finance such work are apparent: 1) grants from the philanthropic foundations, 2) governmental aid, 3) institutional funds, 4) direct grants from commercial companies.

Since early 1940 various of the philanthropic foundations have increased their interest in tropical medicine or have entered the field for the first time.

Emergency aid to strengthen the teaching of tropical medicine in medical schools has been provided, notably by the Markle Foundation. Large-sized grants for the basic strengthening of departments of tropical medicine have been made; for example, the Rockefeller Foundation's \$200,000 gift to Tulane in 1941 and the Macy Foundation's recent gift of \$150,000 to Columbia University's College of Physicians and Surgeons.

These and other grants indicate a shift in the interest of philanthropic foundations from research and preventive work to the strengthening of facilities for a better preparation of medical students in tropical medicine. The very breadth of foundation interest prior to 1940 may have contributed to what many felt to be a basic weakness so far as tropical medicine within this country is concerned. Although large contributions had for years been made toward research and sanitation projects in many quarters of the globe, very few funds had been devoted to developing in this hemisphere good facilities for the teaching of tropical medicine.

The consequences in terms of the lack of well-trained physicians for the armed forces are well known to you all, as are the corrective steps taken through the enlargement of facilities at the Army Medical School and expansion of training opportunities at Tulane.

Will the philanthropic foundations continue their interest in the teaching of tropical medicine when the war emergency is over? Some medical schools have made large commitments in men and facilities. These must continue to be financed. The philanthropic foundations have been the most important private source of funds for wartime expansion of teaching. Without doubt they appreciate that a part of the responsibility for continuing support is theirs.

Reference has already been made to the tropical medicine teaching at the Army Medical School. This work and the instruction given by the Navy to its medical corps officers has constituted an important contribution to the problem by governmental agencies. Staff members of medical schools as well as medical corps officers have taken the Army course. Among its other wide interests, the Office of the Coordinator of Inter-American Affairs financed the fellowship for Latin American physicians enrolled in the postgraduate course at Tulane.

Through the National Research Council a large amount of public funds has been allocated to numerous institutions for fundamental investiga-

tion in the field of tropical diseases, most of it confidential in nature. The Council has been used as an agent by one foundation which has financed the distribution of teaching materials and the services of travelling lecturers for medical students.

Will public funds be available for teaching tropical medicine after the war? Probably not on the present scale; perhaps not even on a much reduced scale; and probably only for purposes which have a bearing on public health.

The two remaining sources of financial support are institutional funds and grants from commercial companies.

Most schools have widened substantially their offerings in tropical medicine. Some of them are perhaps financing this out of increased income due to the speedup of the medical course. Others may have secured special gifts from individuals or corporations. Others have simply had one of their teaching staff devote more instructional time to the subject.

Whatever the source of funds, it seems clear that any allocation to tropical medicine instruction from inflated medical school incomes is of an emergency nature. After the war, shrinking income will meet expanding demands for graduate and postgraduate training in the basic fields of private practise. How much will be left over to consolidate the growth of wartime tropical medicine is problematical.

Direct grants to medical schools from commercial companies do not yet constitute an important source of financial support for the teaching of tropical medicine. Their interest is naturally in research on and development of saleable products, much of it done in their own research laboratories. An increasing number of companies are supporting the teaching of tropical medicine through their gifts to the American Foundation for Tropical Medicine.

This brief review of the main sources of financial support for tropical medicine may serve to point up one of the problems which the Society and other interested groups should be and doubtless are considering. When the wartime honeymoon is over, what agencies will rally to support the expanded interest in tropical medicine in American medical schools?

### III

The American Foundation for Tropical Medicine has a major opportunity here. It can become one of the prime agencies to finance the consolidation of

recent advances in the teaching of tropical medicine.

The Foundation's potentialities for growth are considerable. Its annual income could be doubled or tripled—*provided* that a qualified man is found who can serve the Foundation full time. Operating costs, which have ranged between three and four per cent since 1940, will necessarily increase under that arrangement. But full time service is essential to full growth. The executive committee is well aware of this fact and is in search of such a man.

\$250,000 to \$300,000 a year devoted to purposes such as the Foundation has aided would help substantially to bridge the gap between war and post-war financing of medical school offerings in tropical medicine and parasitology and for related work.

A *modus operandi* for the Foundation has been established. The Foundation has demonstrated its usefulness as a source of supplementary funds. Its medical committee of seven members reviews each application. This fact gives a measure of assurance of the maintenance of high standards, both in the selection of projects and the conduct of the work. Organized to serve the field of tropical medicine, it is on the way to becoming the important factor in the field which its founders envisioned. An increasing number of corporations are finding it a valuable means through which to express their interest in building up this country's resources in tropical medicine, in terms of trained teachers, good research men and adequate facilities for both.

Dr. Alfred C. Reed,<sup>4</sup> a member of the Foundation's medical committee, speaking at the Memphis

<sup>4</sup>Reed, A. C., "The Future of Tropical Medicine." *American Journal of Tropical Medicine*, 20, 1-11 (Jan. 1940).

meeting of this Society four years ago, quoted some remarks of Dr. McKinley's which I should like to cite in concluding. Dr. Reed notes that Dr. McKinley constantly emphasized the permanent importance of the tropics in the world economy and the future relations of the United States.

"We hope that the time will come," said Dr. McKinley, "when several basic industries will support a public health program in the tropical countries in which they invest their capital, as one contributing form of insurance for the success of their enterprise."

A number of commercial companies have gone far in the development of medical facilities in tropical areas. But to build hospitals and treatment stations and to take preventive measures is one thing. To find physicians, other scientists and technicians trained in the treatment and control of tropical diseases is quite another. It is no coincidence that the United Fruit Company and Firestone Plantations Company are among the most generous supporters of the Foundation. They realize the importance of good facilities for basic training in tropical medicine *in this hemisphere* and the necessity of assuring a constant flow of trained personnel for their own and for other establishments.

As this country's commitments in tropical areas increase, we must develop the power to fulfil our commitments, working in cooperation with the authorities of each area. Power, in terms of health, means facilities and personnel adequate to grapple with any health problem we may find in any quarter of the globe.

By strong measures the wartime needs of our armed forces in this field are being met. Equally strong measures are called for to meet the needs of the postwar period.

## BOOK REVIEWS

*Medical Parasitology and Zoology.* By RALPH WELTY NAUSS, V.Sc., M.D., Dr.P.H., Asst. Professor of Preventive Medicine and Public Health, Cornell Univ. Medical College. Pp. i-xiv, 1-534, Figs. 1-95, 1 Color Plate. First Edition, Paul B. Hoeber, Inc., New York and London. 1943.

A reviewer of any book should discover first of all the purpose of the author in placing his information on record, and should then proceed to discover if that objective has been fulfilled. In the Foreword by Professor John C. Torrey and in the Preface there are statements indicating that this volume is the outgrowth of experience in teaching second-year medical students in the department of public health and preventive medicine, Cornell University Medical College, New York City. In this institution the subject of medical parasitology is allotted ten sessions of three hours each, during which both laboratory and lecture methods of presentation are employed. The book contains not only the material which can be presented in the limited time available, but a substantial amount of collateral reading.

The volume is divided into four parts, namely, (1) Protozoa, (2) Parasitic Worms, (3) Anthropods and Disease Transmission, and (4) Poisonous and Venomous Forms, and is supplemented by nine chapters headed "Appendices," a glossary, bibliography and subject index. For each important parasitic infection considered there is a description of the etiologic agent, its life cycle, epidemiology, pathogenesis, symptomatology, diagnosis, treatment, prognosis and prophylaxis. On the whole there is abundant information of interest and value to the medical student.

Chapter I, on the intestinal amebae, takes up these organisms first of all from a laboratory point of view. The reviewer feels that the author would have been wise in recommending 1½ by 3 inch rather than one by 3 inch microscopic slides, and D'Antoni's iodine rather than Lugol's solution for staining fresh fecal preparations. Table I (Amebae: The living Trophozoites) refers to the genera of amebae by initial only (i.e., *E. histolytica*) seven pages previous to any full name or description of the species considered. Tables II and III also precede the first textual description of the organisms. This is not likely to be lucid to the average preclinical student. In Chapter II (Amebiasis), page 25, "amebic dysentery" is defined as "characterized by a bloody mucoid diarrhea," a conception most difficult to comprehend, since dysentery is usually understood to provide a stool consisting essentially of blood, mucus and tissue detritus, while *diarrhea* is merely *unformed fecal matter*, at times with mucous and tissue-cells. Fig. 7 (page 39), illustrating "cell picture in the feces of acute amebic dysentery," shows an ameba, an intact

leukocyte, a few erythrocytes, several disintegrating nuclei of tissue cells, bacteria, Charcot-Leyden crystals (not necessarily present in amebic dysentery), but no feces. Under the heading of treatment for amebiasis (page 42) chiniofon, anayodin and yatren may be inferred to be as distinct from one another as they are from vioform. The dosage is (inadvertently?) given as "3 or 4 enteric-coated 4 grain (0.25 gm.) pills or tablets daily," rather than the amount to be administered t.i.d. Diodoquin is not mentioned, although emetine-bismuth iodide, which most American physicians discarded twenty years ago, is allotted a separate paragraph.

In Chapter III (page 61) *Triatoma megista* is stated to be "the principal transmitting insect agent" of Chagas' Disease, whereas *T. infestans* is a much more prevalent vector. In the treatment of kala-azar (page 66) the author recommends "antimony in the form of the double tartrate of sodium and potassium (tartar emetic)." Possibly the author refers to the older preparation consisting of a mixture of sodium antimony tartrate and potassium antimony tartrate, but today these tartrates are available separately and are prescribed as such. In Chagas' disease (page 78) reference is made to the division of *Trypanosoma cruzi* in the leishmania stage "in tissue cells, especially of the striated and heart muscle." This host-cell predilection is probably much less common than that of the reticulo-endothelial cells of the spleen, liver, bone marrow or even the neuroglia cells.

The two chapters on Sporozoa and Malaria Fevers (V and VI) are well written and contain much usable information.

In the section on parasitic worms, consideration is first given (Chapter VIII) to those roundworms which are propagated by soil contamination. The first worm considered (*Enterobius vermicularis*) is not propagated primarily as a result of soil pollution but is rather a household infection, especially in the sleeping quarters. The otherwise excellent diagram illustrating the life cycle of this worm (page 139) is also similarly in error. In the treatment of oxyuriasis hexylresorcinol crystals (page 140) are recommended as the drug of choice, although four-hour Seal-Ins or Enseals gentian violet med. is now known to be much more satisfactory. The author fails to mention that treatment will not be effective unless all parasitized members of a family are given the anthelmintic simultaneously. *Trichuris trichiura* is the name utilized for the human whipworm but difficulty has apparently been experienced in the spelling, since different orthography is found at several places in the text and appendix. In ascariasis (page 146) the diagram of the life cycle of the etiologic agent omits the most common mechanism of infection, namely



accidental direct transfer of infective-stage eggs from the ground to the mouth of children playing on contaminated soil.

The consideration of hookworm infection is on the whole well written. However, fig. 35 (page 152) is incorrect in showing the copulatory spicules of the male *Necator* as separated distally; they should be figured as fused and tipped with a single barb. In the treatment of hookworm infection hexylresorcinol crystoids are recommended. (See also a similar reference for oxyuriasis, page 140). In the United States the trade name of this preparation is caprokol. For strongyloidiasis (page 168) mention is not made that gentian violet should be prescribed as medicinal in one and one-half or two hour Seals-Ins or Enseals coating.

Chapter IX (Food-Infesting Worms) considers *Trichinella spiralis* and several flukes and tapeworms which man acquires from consuming inadequately processed food. The treatment on *Trichinella* and trichinosis is excellent, particularly because it presents much original work of the author. In considering the giant intestinal fluke, *Fasciolopsis buski* (page 186), no mention is made of the clinical study of McCoy and Chu (1937) indicating the high efficiency of caprokol (hexylresorcinol crystoids). Exception is taken by the reviewer to the expression "*Taenias, saginata* and *solum*" (pages 192, 195) in place of "*Taenia saginata* and *T. solium*." Moreover, it is difficult to understand how *Echinococcus granulosus* (page 201) and *Hymenolepis nana* (page 206) can be considered as "food-infesting worms" in so far as man is concerned.

In Chapter X (Filaria and the Filariases), page 215 et seq., the generic name *Wuchereria* is spelled incorrectly with an umlaut *ü*, and on page 221 the species named "malayi" is misspelled "malaya." In Table XII (page 234) an old-fashioned nomenclature (ante-1924) is used for the species of snails serving as intermediate hosts of the Oriental blood fluke, *Schistosoma japonicum*.

In Table XIII (pages 246-247) in the introductory chapter to Arthropods and Disease Transmission twelve technical and typographical errors occur. The map (Fig. 66, page 266) on the geographical distribution of yellow fever includes former epidemic foci in Mexico and Central America which have been free of the scourge for two decades, while the accompanying map (Fig. 67, page 267) on the distribution of the yellow fever mosquito (*Aedes aegypti*) does not include extensive areas in Central China where this mosquito breeds. On page 316 "murine typhus" and "Brill's disease" are used synonymously. This is not justified on the basis of recent serological tests. Many of the technical terms and some of the statements in this section of the book require a critical revision.

Part IV (Poisonous and Venomous Forms), pages 333-374, is an interesting section, because it includes the venomous snakes and management of snake venenation.

Appendices I-IX (pages 377-430) contain information for constructing a simple warming stage for the microscope, care and use of the microscope, "pseudomorphs" in stools, staining and culture methods for protozoa, the NIH anal swab technic for recovering eggs of seatworms, concentration technics for protozoan cysts and helminth eggs passed in feces, collection and preservation of parasitologic material, and summary classification and distribution tables of venomous snakes of the Western Hemisphere. Glossary I (pages 431-438) is entitled "Classification of Animal Parasites and Anthropods" (*sic*), while Glossary II (pages 439-479) is a lengthy list of terms alphabetically arranged, with their definitions and derivations. There is a "Bibliography" (pages 480-488) and an "Additional Bibliography" (pages 488-497). The reviewer has been unable to discover any reason for this division of references.

The volume is clearly printed and attractively bound and contains 95 figures and a beautiful color plate on the malaria parasites. Only a few of the figures are original. Some of the engravings are inaccurate, some have been reduced too much and others are poorly reproduced.

With the errors and inconsistencies which have been noted, the reader might possibly believe that this volume on Medical Parasitology and Zoology is not a valuable contribution to the subject. Such is not the case. Although it is subject to the difficulties of many a first edition, there is an intelligent consideration of the material, much of which will be helpful to medical students. But until a second printing or a new edition can be brought out to adjust and rectify these inconsistencies it is suggested that the reader check the technical information either against original sources or some other modern treatise on the particular subject.—ERNEST CARROLL FAUST.

*The Principles and Practice of Tropical Medicine.* By L. EVERHARD NAPIER, C.I.E., F.R.C.P., Director and Professor of Tropical Medicine, Calcutta School of Tropical Medicine and Senior Physician Carmichael Hospital for Tropical Diseases, Calcutta, India. Pp. I-XII, I-522. Thacker, Spink and Co. Ltd., Calcutta and London. 1943.

The clinician will welcome this new work upon tropical medicine as it is written by a noted specialist especially for the clinician. The complete work will be published in two volumes, the second to appear later, and this volume considers the following subjects: Malaria, Leishmaniasis, Trypanosomiasis, the Relapsing Fevers, Rat Bite Fever, Leptospirosis, the Typhus Fevers, Oroya Fever, Yellow Fever, Rift Valley Fever, Dengue Sand-Fly Group, Plague, Tularemia, the Undulant Fevers, Melioidosis, the Intestinal Fluxes and Leprosy. The second volume will consider Yaws, Tropical Ulcerative Conditions, Helminthic Infections,

Snakes and Snake-Bite, Anemias of the Tropics and Diet and Dietetic Diseases.

It is impossible in a review to give an adequate analysis of this important work but it may be stated that the chapters upon malaria, kala-azar, plague and cholera contain the best clinical descriptions of these infections that the reviewer has seen within recent years and are especially valuable from the stand-point of treatment. Dr. Napier is a world-authority upon kala-azar and a discussion of this infection from his pen would be expected to be a most valuable one and the reader will not be disappointed in this respect. The discussion of the treatment of cholera is also most excellent and throughout the work clinical symptomatology is stressed and the clinical descriptions are clear and most valuable. The author is apparently much concerned over what he terms "the danger of the complete laboratory domination of tropical practice" but it would appear that this concern is really unwarranted unless he means by this domination the dependence of tropical medicine upon the laboratory diagnosis of the infections encountered in the tropics. It is undoubtedly true that in this respect the laboratory must dominate tropical practice, for it so happens that the vast majority of the infections encountered in the tropics and sub-tropics must depend upon the laboratory demonstration of their etiological agents in diagnosis for one cannot depend upon a clinical diagnosis in such infections. This is a condition that must be faced by the clinician and he cannot help but admit that in this respect the laboratory does, and will continue to, dominate the practice of medicine in the tropics.

It is unfortunate that the author will not accept the term "Amebiasis" in discussing infection with *Endamoeba histolytica* and insists upon considering this important infection under the term "Amoebic Dysentery." A review is not the proper place to thoroughly discuss this subject but it may be stated that in thus adhering to this out-dated conception of this infection the author stands practically alone at the present time. It is also evident that he will not change his opinion no matter how cogent the reasons for doing so, as he states: "There are many parallel examples which the writer could quote in favor of his point of view and, even if there are as many that could be quoted against it, he still proposes to adhere to his view, as he considers that the adoption of this classification will help to rescue tropical medicine from laboratory domination." The author's conception that the primary amoebic infection should be called "Amoebic dysentery" and that all the other symptoms which are present should be considered as secondary to dysentery is certainly unique and not held by the vast majority of students of the subject. Also his belief that the symptom free "carrier" is not a danger in the transmission of the infection completely ignores all of the evidence furnished by the work of Faust, Meleney and others, demonstrating that the various strains of *Endamoeba histolytica* occurring in

symptomless carriers are pathogenic and capable of causing dysentery, although variations do occur between different strains, but no strain from such carriers has been proven to be non-pathogenic. In fact, the author fails to mention most of the decisive and extensive work upon amebiasis contributed by American observers, possibly because of his fear of the laboratory domination of the subject. In the discussion of treatment no mention is made of diodoquin and the author still adheres to the term "*Entamoeba histolytica*" instead of "*Endamoeba histolytica*" although the former spelling is against the rules of the International Commission on Zoological Nomenclature. The list of references for this chapter is very limited and, with only three exceptions, no recent papers upon amebiasis are included.

However, taken as a whole, this work upon tropical medicine is one of the best that the reviewer has seen. It is essentially a book written by a clinician for the clinician and in no sense a work of reference. The descriptions of disease are excellent from the clinical standpoint and the book can be recommended without hesitation to the physician in tropical and other regions where the various disease conditions discussed may occur.

The book is well printed and bound and illustrated with eighteen full-page plates, three of which are colored, and one hundred and thirty-eight cuts.—CHARLES F. CRAIG.

*Handbook of Health.* By GEORGE CHEEVER SHATTUCK, M.D., AND WILLIAM JASON MINTER, M.D. Second Edition, Revised. Pp. I-VII, 1-228. Harvard University Press. 1943.

This little book is intended "for use by persons overseas who are engaged in more or less dangerous work and who, from time to time, may be unable to obtain medical advice or assistance" and it well fulfills its mission. It is written in plain, simple language which can be understood by the average individual and treats of the following subjects: Keeping Fit, Common Ailments of World-Wide Occurrence, The Tropics, Diseases Important in the Tropics, Biting Insects, Vermin and Snakes, The Arctic, Surgery, First Aid, and contains an Appendix in which are considered Packing Medical Supplies and a Table of Weights and Measures.

A careful reading of this book has convinced the reviewer that it furnishes a most valuable source of information on the subjects treated for those for whom the work is intended and he has no hesitation in recommending it as the best work of its kind that has come to his attention. The volume is of pocket-size, and is well printed and bound and suitably illustrated.—CHAS. F. CRAIG.

*A Manual of Medical Parasitology.* By CLAY G. HERR, Professor of Parasitology, University of Chicago. Pp. I-X, 1-88. University of Chicago Press. 1943. This small manual upon medical parasitology may

prove useful to the classes conducted by the author at the University of Chicago but it is doubtful if it will be as useful to the general student of medicine, as it considers the entire subject in the very limited space of 88 pages, which cannot be done and give the student any real conception of the importance of parasitology to the medical man. The reviewer cannot conscientiously recommend this manual for, in his opinion, it is inadequate as a teaching manual and would give the student a very limited knowledge of the many important parasites causing disease in man-kind.

The surprising statement is made in the Preface that "one of the greatest deficiencies felt in presenting the laboratory work has been the lack of colored figures of the malarial parasites, which would make possible the rapid identification of the various forms in blood-films." Surely the author must know that practically every work upon tropical medicine and parasitology contains most excellent colored plates illustrating the malaria plasmodia. The plate printed in this manual is excellent but no better than numerous others contained in the works above mentioned.—CHAS. F. CRAIG.

*Clinical Diagnosis by Laboratory Examinations.* By JOHN A. KOLMER. D. Appleton-Century Co. New York and London. 1943.

Dr. Kolmer has brought together much interesting material in this large volume of 1239 pages. More than half of the book is devoted to the clinical interpretation of laboratory examinations. The remainder is given over to the practical application of laboratory examinations in clinical diagnosis, to the technic of such examinations and to a 130 page index. Many tabular out-lines appear, particularly in the first half of the book. Colored plates give excellent impressions of the various reactions to different skin tests but the important color characteristics of the various malaria plasmodia are lost in black and white. Selected references to the literature are grouped at the conclusion of chapters.

Beginning with the clearly stated premise "No clinical or laboratory examination can be better than the thoroughness and skill with which it is conducted"

this work is a guide to the just estimate and sound utilization of laboratory data which alone give it honest clinical value. The best chapters are those devoted to serological tests and their interpretation. Other chapters contain less original material and more material from other standard works. Typographical errors are not rare, e.g., atomy, page 267; Chaga's, p. 296; L. ictero hemorrhagia, p. 351; S. japonica, p. 777; X. cheopsis, p. 946; E. granulosis, p. 1057 and others.

To summarize, one finds in this book many useful facts selected by an author of wide experience but it is a work of general reference rather than an essential "buy" for the average physician or hospital.—ELLISTON FARRELL.

*The Organization of Permanent Nation-wide Anti-Aedes aegypti Measures in Brazil.* By FRED L. SOPER, D. BRUCE WILSON, SERVULO LIMA and WALDEMAR SA ANTUNAS. Pp. 1-137. Illustrated. The Rockefeller Foundation, New York. 1943.

This report of the operation of an *Aedes aegypti* campaign is published by the Rockefeller Foundation at the request of Sir Malcolm Watson, who, after observing the methods employed by the Brazilian Government, in conjunction with the Foundation, urgently requested that the methods used be published "so that in other countries men who are faced with the problem of mosquito control will have this invaluable guide."

The report embodies a complete and detailed description of the methods employed by the Brazilian Government and the Foundation in the control of *Aedes aegypti* in Brazil, which was undertaken to prevent the spread of yellow fever in that country. The campaign was eminently successful and this report contains copies of all of the various blanks, charts, etc., used, together with the regulations regarding inspections and the various methods employed for the destruction of this mosquito. It is a work that should be in the hands of every health officer, and all who are concerned in anti-mosquito campaigns, and the Rockefeller Foundation has performed a notable public service in making it available to the sanitarian and public health physician.—CHARLES F. CRAIG.

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MANUAL ON THE DISTRIBUTION OF COMMUNICABLE DISEASES AND  
THEIR VECTORS IN THE TROPICS\*

PACIFIC ISLANDS SECTION—PART I

EDWARD PHILPOT MUMFORD AND JOHN LUTHER MOHR<sup>1</sup>

*Stanford University*

The present manual is a wartime outgrowth of Hawaii Board of Health and the Hawaii Territorial

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BY

EDWARD PHILPOT MUMFORD AND JOHN LUTHER MOHR

*Stanford University*

INTRODUCTION

DISEASES OF DOUBTFUL ETIOLOGY

DISEASES CAUSED BY FILTERABLE VIRUSES, RICKETTSIAE  
AND ALLIED ORGANISMS

DISEASES CAUSED BY BACTERIA

DISEASES CAUSED BY FUNGI

DISEASES CAUSED BY SPIROCHAETES

DISEASES CAUSED BY PROTOZOA

DISEASES CAUSED BY METAZOA

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*Bacto-Russell Double Sugar Agar*

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# MANUAL ON THE DISTRIBUTION OF COMMUNICABLE DISEASES AND THEIR VECTORS IN THE TROPICS\*

## PACIFIC ISLANDS SECTION—PART I

EDWARD PHILPOT MUMFORD AND JOHN LUTHER MOHR<sup>1</sup>

*Stanford University*

The present manual is a wartime outgrowth of the senior author's work in the Pacific Islands Research project carried on at Stanford University since 1939.

Publication of the material in its present form is made in response to urgent requests. Revised editions will be issued from time to time, and final publication in book form will include references to the extensive source material consulted. This includes official reports, occasional articles, medical and anthropological, and extensive correspondence and discussion. Suggestions for the expanded future editions and final publication in book form will be appreciated. Observations by physicians now stationed in this area are particularly welcome.

The Pacific section, of which this is the first part, is being prepared in consultation with Dr. S. M. Lambert, formerly Rockefeller Foundation medical representative in the Pacific. Another advisor has been Dr. V. W. T. McCusky, Director of Medical Services, Fiji and the Western Pacific. Valuable recent data have been received from the

Hawaii Board of Health and the Hawaii Territorial Medical Association. Acknowledgement is made to Dr. Ray Lyman Wilbur, Chancellor of Stanford University and Trustee of the Institute of Pacific Relations; Sir Maynard Hedstrom, formerly of the Executive Council, Fiji Crown Colony; and Sir Philip Mitchell, Governor of Fiji and High Commissioner for the Western Pacific. Chancellor Wilbur and Sir Maynard Hedstrom have been unfailing sources of encouragement and support. Sir Philip Mitchell's specially appointed committee of medical officers made helpful suggestions and additions.

The need for such a Pacific study and supplementary technical papers is indicated by the fact that the most recent comprehensive work on diseases distribution, the late Dr. E. B. McKinley's *Geography of Disease*, 1935, makes no mention of such regions as the Japanese Mandated Islands, New Guinea, Papua, and the New Hebrides. Under the auspices of the Macy and other Foundations, the writers have published a preliminary report on the parasitic and other infectious diseases of the Japanese Mandated Islands (*Amer. J. Trop. Med.*, Vol. 23, No. 4, July, 1943). Similar technical papers will be issued for other Pacific Island areas not included in McKinley's study.

The first part of the Pacific section, here following, discusses the materials by disease. Subsequent sections to be issued as supplements will discuss more extensive material, including arthropod vectors, geographically. The arrangement of the distribution records is uniform, in general from West to East.

A table of distribution is included. In the table it should be borne in mind that many of the records (e.g. all those for smallpox) are old and some are almost certainly allochthonous (e.g. for Clonorchiasis). The islands groups in the heading are taken from *Pacific Islands Yearbook*, War-Time Edition, 1942, Sydney, and are used as defined there.

\* The investigation has been aided by grants from the Josiah Macy Jr. Foundation.

<sup>1</sup> Among those who have read the manuscript and made helpful suggestions, special mention may be made of Chancellor Ray Lyman Wilbur and Professors O. N. Andersen, G. F. Ferris, G. S. Lockett, S. Raffles, E. W. Schultz, and Alfred C. Reed, of Stanford University; Professors W. B. Herms and Gordon H. Ball, of the University of California; Dr. Wilbur A. Sawyer, Director of International Health Division, Rockefeller Foundation; Capt. T. J. Carter, Lt. Comdr. D. F. Smiley, and Lt. (j.g.) D. S. Farner, Bureau of Medicine and Surgery, Department of Navy; Capt. A. P. Krueger, U.S.N.R., Laboratory Research Unit No. 1; Dr. K. F. Meyer, Director of Hooper Foundation, University of California; Prof. E. C. Faust, acting head, Department of Tropical Medicine, Tulane University; and Dr. J. E. Alicata, Director of the Parasitology Project, Public Health Committee, Territory of Hawaii.



TABLE 1  
Distribution of Communicable Diseases in the Pacific

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Cook Islands	Dutch New Guinea	Easter Island	Fiji Islands	French Oceania	Gilbert and Ellice Islands	Guam	Hawaiian Islands	Japanese Mandated Islands	Nauru	New Caledonia	New Guinea Territory	New Hebrides	Niue	Papua	Pitcairn Island	Samoa—American	Samoa—Western	Solomon Islands Protectorate—British	Tonga Islands
Actinomycosis.....				x				x			x	x	x					x		x
Amoebiasis.....				x		x	x	x	x		x	x	x		x		x	x	x	
Ancylostomiasis.....	x	x		x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x
Anthrax.....								x	x		x				x					
Ascariasis.....	x			x	x		x	x	x	x	x	x	x	x	x		x	x	x	x
Bacillary dysentery.....	x	x		x		x	x	x	x	x	x	x	x		x		x	x	x	
Balantidiasis.....				x			x		x						x			x		
Blackwater fever.....		x										x	x		x				x	
Blastomycosis.....															x					
Cerebrospinal meningitis, epidemic.....				x			x	x	x	x	x	x	x	x	x				x	
Chancroid.....				x	x		x	x	x	x		x						x	x	
Chicken pox.....	x	x		x		x	x	x	x	x	x	x	x	x	x		x	x	x	x
Clonorchiasis.....								?	?			?							?	
Cold, febrile.....	x			x		x	x	x	x	x				x	x	x	x	x	x	
Dengue fever.....	x			x	x	x	x	x	x		x	x	x		x		x	x	x	x
Diphtheria.....		x		x		x	x	x	x		x	x	x		x				x	x
Diphyllobothriasis.....															x					
Encephalitis, epidemic lethargica.....				x		x		x												x
Erysipelas.....	x			x		x	x	x	x	x							x	x	x	
Erythrasma.....								x	x	x	x									
Fascioliasis.....								x	x											
Filariasis.....	x	x		x	x	x	x		x	x	x	x	x	x	x		x	x	x	x
German measles.....	x			x			x	x				x	x		x				x	x
Gonorrhea.....	x	x		x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x
Granuloma inguinale.....		x									x	x	x		x				x	
Hepatitis, acute infectious.....						x		x					x					x		
Herpes zoster.....				x		x			x	x										
Heterophyiasis.....								x												
Hymenolepis infection.....				x				x		x		x	x		x					
Impetigo.....	x			x	x	x	x	x		x	x	x		x				x	x	
Influenza, epidemic.....	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Leprosy.....	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x
Lymphogranuloma venereum.....									x			x	x							
Malaria.....		x																		
Falciparum.....		x										x	x		x				x	
Malariae.....		x										x	x		x				x	
Vivax.....		x										x	x		x				x	
Measles.....	x	x		x	x	x	x	x	x		x	x	x		x	x	x	x	x	x
Molluscum contagiosum.....									x			x						x		
Monihasis (Soor).....								x	x											
Mumps.....	x			x	x	x	x	x	x	x	x	x						x		x
Mycetoma.....								x	x										x	
Mysiasis.....												x							x	
Oxyuriasis.....	x			x		x	x	x	x	x	x	x	x	x	x			x		x
Pappataci fever.....											?	?			?					
Paragonimiasis.....									x	x		x					x	x		

TABLE 1—Continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Cook Islands	Dutch New Guinea	Easter Island	Fiji Islands	French Oceania	Gilbert and Ellice Islands	Guam	Hawaiian Islands	Japanese Mandated Islands	Nauru	New Caledonia	New Guinea Territory	New Hebrides	Niue	Papua	Pitcairn Island	Samoa—American	Samoa—Western	Solomon Islands Protectorate—British	Tonga Islands
Paratyphoid fever.....	x			x			x	x	x		x	x	x		x			x	x	
Plague.....								x			x	x								
Pneumonia, bronchial.....				x		x	x	x		x	x	x	x	x	x		x		x	
Pneumonia, lobar.....				x		x	x	x		x			x	x			x	x	x	
Pneumonitis, Bowman's.....								x												
Polio-myelitis, acute anterior....				x		x	x	x	x	x		x		x	x			x	x	
Pyomyositis, tropical.....	x			x		x						x	x	x	x			x	x	
Ratbite fever.....				x				x			x							x	x	x
Relapsing fever.....											x									
Rheumatic disorders.....	x			x		x	x	x	x	x		x			x			x	x	
Scabies (and other acariases)...	x			x		x		x	x	x	x	x	x	x	x	x		x	x	
Scarlet fever.....				x			x	x		x	x		x							
Septic sore throat.....				x																
Small pox.....		x	x		x		x	x	x			x								
Sporotrichosis.....								x		x										
Strongyloidiasis.....				x			x	x			x	x	x	x	x		x		x	
Syphilis.....	x		x	x	x			x	x		x		x		x					
Taeniasis saginata.....				x				x	x			x								
Taeniasis solium.....								x		x								x		
Tetanus.....	x		x	x	x	x	x	x	x		x	x	x		x	x	x	x	x	x
Tinea alba.....								x									x	x	x	
Tinea albigena.....		x							x			x						x		
Tinea barbae.....								x											x	
Tinea circinata.....						x		x	x			x		x				x		x
Tinea cruris (Dhobie's itch).....				x			x	x	x			x	x		x		x		x	
Tinea imbricata (Tokelau).....	x	x		x	x	x			x	x	x	x	x	x	x		x	x	x	x
Tinea interdigitale.....				x				x	x									x		
Tinea nigra.....																	x			
Tinea versicolor.....				x	x	x		x	x	x	x	x		x			x		x	
Tonsillitis, acute.....	x			x		x	x	x		x				x	x		x		x	
Trachoma.....	x			x		x	x	x	x	x		x	x		x		x	x	x	x
Trench fever.....				x																
Trichinosis.....								x												
Trichostrongylus infection.....				x																
Trichuriasis.....	x			x	x	x	x	x	x		x	x	x		x		x	x	x	x
Tropical ulcer.....		x		x		x					x	x	x		x			x	x	
Tuberculosis.....	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Typhoid fever.....	x			x	x	x	x	x	x	x	x	x		x	x		x	x		x
Typhus, murine.....								x												
Typhus, scrub.....									x			x			x					
Undulant fever.....				x				x	x				x							
Weill's disease.....								x	x											
Whooping cough.....	x			x	x	x	x	x	x		x	x	x		x	x	x	x	x	
Yaws.....	x	x		x	x	x	x		x	x	x	x	x	x	x		x	x	x	x

## DISEASES OF DOUBTFUL ETIOLOGY

*Acute Infectious Hepatitis (see Leptospirosis)**(Bowman's pneumonitis) Oahu fever*

There occurs in the Hawaiian archipelago a primary atypical or non-specific pneumonia of unknown etiology called Oahu fever or Bowman's pneumonitis, cases of which are not reportable; it reaches epidemic proportions in the spring.

*German measles (Rubella)*

German measles occurs sporadically in New Guinea Territory and Papua. It is reported from Guam, the British Solomon Islands Protectorate, the New Hebrides, Fiji, Tonga, and the Cook Islands. From Hawaii it has been reported separately from measles from 1939-1940 to date although mention was made of it in connection with an epidemic in 1936-1937 and perhaps other years. In most years it is present in all the Hawaiian Islands. 297 cases were reported in 1942-1943.

*Granuloma inguinale (Ulcerating granuloma, Granuloma venereum)*

*Granuloma venereum* is widespread; about 6,000 cases were reported as treated in Dutch New Guinea in 1923-1926. In 1935 the disease was reported as limited to a region lying on the south-east of Digoel River, including Frederik Hendrik Island, and extending as far as the Australian boundary. In New Guinea Territory, it is reported from north-east New Guinea, New Britain, New Ireland, and the Admiralties. Cases, some fatal, are reported from the Territory of Papua and Samarai Island. It occurs in the Solomons. In 1920 it was described from New Caledonia. It is reported from the New Hebrides and Western Samoa. One off-shiping case, confirmed microscopically, was reported in Hawaii in 1942.

*Rheumatic fever and "Rheumatism"*

Rheumatic disorders, particularly acute joint inflammation, are reported from New Guinea Territory; they occur there in north-east New Guinea, New Britain, and New Ireland. In the Territory of Papua, rheumatic fever and rheumatism are reported on the mainland and on Woodlark and Samarai Islands.

Rheumatic fever is reported from Guam. Muscle and joint rheumatism is found in the Carolines and the Marshalls, and rheumatism occurs in the Marianas.

Rheumatic fever and rheumatism are reported on Nauru. Rheumatic fever is reported from the Solomons. Rheumatic fever and acute and chronic rheumatism are reported from the Gilbert and Ellice Islands. Rheumatic fever and acute and subacute rheumatism are reported from Fiji. Rheumatic fever occurs in Western Samoa and the Cook Islands.

Cases of rheumatic fever, some fatal, are reported from Hawaii.

*Tropical ulcer (Tropical sloughing phagedena)*

In New Guinea Territory and Papua the morbidity rate from tropical ulcers is the highest of any disease. In New Guinea Territory in 1936 tropical ulcer was reported as incapacitating 10% of the native plantation workers. It is said to be more virulent in some districts of New Guinea than in others. In Rabaul, New Britain, for example, it is particularly virulent. In the western portion of the Central Division of Papua, on the other hand, less than 1% of the total population was infected in 1935.

McKinley reports tropical ulcer as of doubtful occurrence in Guam. In the British Solomon Islands Protectorate, it is widespread and reaches its highest incidence on Malaita. It is reported from the New Hebrides and the Gilbert and Ellice Islands.

In Fiji in 1933 the incidence is reported to have "assumed almost epidemic proportions." Tropical ulcer occurs in the Wallis and Horn Islands, but it is said not to be present in Hawaii.

DISEASES CAUSED BY FILTERABLE VIRUSES,  
RICKETTSIAE, AND ALLIED  
ORGANISMS*Chicken pox (Varicella)*

Chicken pox is prevalent but mild in Dutch New Guinea. It is endemic in all districts of New Guinea Territory, occasionally epidemic but always mild. In New Guinea Territory it is reported from the mainland, New Britain, New Ireland, and Bougainville Island. In the Territory of Papua it is reported on the mainland and from Samarai Island.

In 1887 chicken pox appeared as a highly fatal epidemic disease in the Marshall Islands of the Japanese Mandated Group. Subsequently the natives appear to have developed resistance to it. Cases have been reported from the Palau, Carolines, and Marianas. It is said to have reached

Guam shortly after the Spanish occupation of the island in the seventeenth century.

Chicken pox is believed by the natives of the British Solomon Islands Protectorate to be an old disease, but Crichtlow believes it to have been introduced there in 1915. Epidemics are reported there, but the disease is not fatal. Chicken pox is reported from New Caledonia and the New Hebrides. Nauru Island has had small epidemics. It is both endemic and epidemic throughout the Gilbert and Ellice Islands. There are occasional epidemics in Fiji and Tonga. It is reported from the Wallis and Horn Islands. In Western Samoa it is prevalent and it is reported present on American Samoa and Niue. In the Cook Islands, outbreaks are reported on Rarotonga, Aitutaki, and Mangaia. It is well known in the Hawaiian Islands where there were 1319 cases reported during the 1938-1939 epidemic and 1447 cases during 1942-1943.

#### *Dengue fever*

Dengue fever has been observed frequently in New Guinea Territory, where it is now endemic. There have been cases there on New Britain and New Ireland. In the Territory of Papua, it is reported from the mainland and Samarai Island.

In the Japanese Mandated Islands, dengue fever has been reported from the Palaus, Marianas, Carolines, and Marshalls. It is reported also on Guam.

Dengue fever was first seen in the Solomons early in 1923; it may have been introduced from New South Wales. It first appeared in New Caledonia in 1884, and there have been a number of epidemics since. It is reported from the Gilbert and Ellice Islands. The disease reached Fiji in 1885 and is now endemic. In July 1943 it was stated that well over 10% of government officials working in Suva at that time had dengue. It is reported from the Wallis and Horn Islands. It was introduced into Tonga from Fiji in 1930. In the same year it slipped into Western and American Samoa from Fiji, though it had been known earlier in Pago Pago, American Samoa. In 1930-1931 there was an outbreak on Puka Puka (Danger Island) in the Cook group.

The earliest reported outbreak of dengue fever in Tahiti occurred in 1846. There have been many others, some lasting almost a year. In Tahiti few inhabitants escaped the epidemic of 1903, although there were no deaths. In the Leeward Islands there were, however, several deaths from dengue

fever. In the Marquesas, dengue appeared for the first time with a ship from Tahiti in February, 1902. Not a native was spared, and those who were weakened by tuberculosis or other chronic diseases died. It reappeared there in December of the same year.

In Hawaii the first recorded dengue outbreak is said to have been in 1903. Cases were reported as recently as 1943. Both *Aedes albopictus* and *A. aegypti* are reported from Hawaii.

*Aedes aegypti*, *Aedes albopictus* and *Culex fatigans* are reported in the Pacific.

#### *Encephalitis, Epidemic lethargic*

Epidemic encephalitis is reported from the Gilbert Islands, Fiji, Tonga, and Hawaii. In the last group about two cases a year are reported.

#### *Febrile colds*

Febrile colds are common in the Territory of Papua. In the Japanese Mandated Islands and Guam they are seasonally prevalent, and may predispose the natives to tuberculosis. They are reported in the British Solomon Islands Protectorate. Severe febrile colds are reported on Nauru. They are reported from the Gilbert and Ellice Islands, Fiji, and Western and American Samoa. They are sometimes severe on Niue. In the Cook Islands they are reported from Tongareva, Rakahanga, and Manihiki and Puka Puka (Danger Island). They also occur on Pitcairn. In the Hawaiian Islands they are considered an important health problem.

#### *Herpes Zoster*

Herpes zoster is reported from Nauru, Gilbert and Ellice Islands, and Fiji.

#### *Influenza*

Influenza epidemics are reported as prevalent along the whole north coast of Dutch New Guinea. The infection is prevalent throughout the New Guinea Territory and is reported there from the mainland, New Britain, New Ireland, the Admiralties, and Buka and Bougainville Islands.

Influenza may have been introduced into Papua along with dysentery. Epidemics have been reported from the D'Entrecasteaux, Woodlark, and Trobriand groups, and Samarai Island.

Influenza is well established in the Japanese Mandated Islands and Guam. In at least some of the islands epidemics are seasonal.

Since the outbreak of the present war, influenza has been reported as epidemic in the Solomon Islands. There is a recent report of malign epidemics with frequent attendant fatal haemorrhagic broncho-pneumonia. The infection is both endemic and epidemic at Noumea, New Caledonia. About 1890 it was introduced into the Loyalties from New Caledonia, where it was raging at that time. It reappeared in the Loyalties in 1901-1902 and mortalities were high. It is frequently epidemic in the New Hebrides also, with high mortality. Nauru has had repeated epidemics. The October, 1920 epidemic of pneumonic influenza there affected almost 100% of the native population, with a mortality of about 30%. Influenza is reported as endemic in the Gilbert group; and epidemics are reported there and in the Ellice Islands.

There are frequent epidemics of influenza in the Fiji Islands. Rotumah, close by, remained free of the disease during the pandemic of 1918. It is reported from Wallis and Horn Islands. Outbreaks of the disease have occurred in Tonga.

In Western Samoa the pandemic of 1918 was fatal to almost a fifth of the native population. It did not reach American Samoa, but milder epidemics have been reported from both groups. There was a violent epidemic of influenza on Niue in 1909, and there have been a number of less severe ones since that time.

In the Cook Islands, influenza is reported from Puka Puka, Rakahanga, Manihiki, and Tongareva in the northern group, and from Raratonga, Mangaia, Mauke, Aitutaki, and Atiu in the southern group.

The influenza virus introduced to Tahiti from San Francisco in November, 1918, seems to have been very virulent, and the natives were highly susceptible to it. The death rate among the natives and half-castes in Papeete, Tahiti, at that time, was over 50%. The Tuamotus, Australs, Marquesas, and Gambiers escaped the pandemic of 1918. A less virulent form of the disease reached the Marquesas toward the end of the last century. Though it is generally mild, there are three or four outbreaks a year. The *Pitcairn Medical Report* records epidemics of influenza on that island. In the Territory of Hawaii, there are always groups of cases of influenza and often large epidemics. The epidemic of 1940-1941, in which 16,818 cases were reported, was of type A influenza which was subsequently epidemic on the mainland, first on the West coast and later in New

York. During the year 1943, 4288 cases have been reported.

*Lymphogranuloma venereum (Climatic bubo, Tropical bubo, Lymphogranuloma inguinale)*

Climatic bubo is reported from New Britain in New Guinea Territory. In Samoa there was an epidemic of this disease in 1883. It is reported from the Carolines. According to McKinley, this disease is absent from Guam, the Solomons, Fiji, American Samoa, and Hawaii. Cases have been reported in the Hawaiian group but have not been shown to be autochthonous.

*Measles (Rubeola)*

Measles is reported as prevalent but mild in Dutch New Guinea. In New Guinea Territory where it has been reported from both the mainland and New Britain there have been epidemics. Cases have occurred in the Territory of Papua.

Measles is widespread in the Japanese Mandated area. It occurs frequently on Guam, and is often fatal.

Measles was introduced into the British Solomon Islands Protectorate in 1914 and 1915, and it is now endemic. It is rarely fatal there. Epidemics have occurred. In New Caledonia measles is frequently epidemic and occasionally fatal. It has been introduced into the Loyalty Islands.

The New Hebrides were among the first of the Pacific Island groups to suffer from measles. In 1861 Aneityum's population was reduced two-thirds by it. Two years later a "labor vessel" from Queensland took the disease to Eromanga Island in the New Hebrides, where some 2,000 people died of measles.

The Gilbert and Ellice Islands suffered one of the few very serious attacks of measles in recent times in 1936, when 14,282 cases, 100 of them fatal, occurred following the visit of an infected ship from Fiji. However, this pales beside the epidemic of 1875, during which measles with dysentery and famine killed 20,000 Fijians, nearly a quarter of the population, within one week. At present the Fijians have some immunity but measles is still a factor with young native children. In Rotumah there was a fatality rate of 21% in 1911. It is now endemic in Tonga and Samoa, in both of which groups epidemics are reported. The natives of the Cook Islands still appear to have low resistance to infection; an outbreak in 1938-1939 was reported as severe on Raratonga, Aitutaki,

Atiu, Tongareva and Manihiki. Measles is now endemic in the Cook Islands. In 1928 there was an epidemic in the Society Islands and the Marquesas. In Tahiti, 1,349 cases were reported in a total population of 9,585. In the Marquesas there were 593 cases in a total population of 2,255. On Pitcairn Island some immunity against measles has been developed.

In Hawaii in 1937 there was an epidemic involving 13,678 cases, 205 of them fatal. Since then, measles has been minimized with 150 cases reported 1942-1943.

#### *Molluscum contagiosum*

*Bungai*, of New Guinea Territory once considered a disease sui generis, has been proved to be *molluscum contagiosum*. The etiology was worked out by Prowazek on a case found by Poleck on Upolu, Western Samoa. *Molluscum contagiosum* is reported as widespread in the Marshall Islands and as occurring in Fiji and the Gilbert and Ellice Islands.

#### *Mumps*

In New Guinea mumps was more common, or at least more often reported, during the German administration, 1884-1914, than it has been in recent times. Cases were then reported from the mainland, New Britain, and New Ireland. There are recent reports from New Britain.

Mumps is reported in the Japanese Mandated Islands. In 1913 there was an epidemic in Guam with 6,320 cases among the natives. This covered over 50% of the native population.

An epidemic of mumps in the Solomon Islands was reported in 1939. An epidemic was reported in the New Hebrides, 1921-1922, although Placidi reported it in 1932 as unknown there. Cases are recorded from Nauru. A few cases of the disease have been reported from the Gilbert and Ellice Islands over a number of years.

Single cases and epidemics of mumps are reported from Fiji. The infection is reported as occasionally epidemic in the Wallis and Horn Islands. It is reported from Tonga. In Western Samoa it is prevalent and common among children. In American Samoa, it was known as early as 1851. Mumps occurs in the Cook Islands, cases having been reported from Rarotonga and Aitutaki Islands. It is reported as epidemic in Tahiti and is endemic and common in Hawaii, where there was an epidemic of 2999 cases, 1938-1939, the largest on record for the Territory.

#### *Pappataci fever (Sandfly fever)*

The distribution of pappataci or three-day fever is usually considered limited to areas in which sand flies of the genus *Flebotomus* abound. In the Pacific Islands area *Flebotomus* is reported from Port Moresby, Papua, where a common mild fever may be "three-day fever," and on Manus Island in the Admiralty group. In 1938 an epidemic of three-day type fever was reported from New Caledonia. These records are very dubious.

#### *Poliomyelitis, acute anterior*

Poliomyelitis occurs sporadically in New Guinea Territory and the Territory of Papua. In New Guinea Territory cases are reported on the Mainland and from New Britain, New Ireland, the Admiralty Islands and the St. Matthias Islands. In the Territory of Papua the disease is reported on the mainland, in the D'Entrecasteaux group, and on Samarai Island.

In the Japanese Mandated Islands, there have been recorded outbreaks which may be considered provisionally as acute anterior poliomyelitis. An important epidemic of the disease is reported from Guam in 1899.

Epidemics of anterior poliomyelitis have been reported in the British Solomon Islands Protectorate. Cases, some of them fatal, are reported from Nauru, where there was an outbreak in 1910. According to the *Tropical Diseases Bulletin* supplement for 1938, poliomyelitis "usually occurs" in the Ellice Islands. A few cases are reported from Fiji. An outbreak of poliomyelitis was reported to have occurred in Western Samoa in 1932. It is reported from Niue, but is said to be absent from the Cook Islands.

Acute anterior poliomyelitis is reported regularly from Hawaii with increasing morbidity rates.

#### *Psittacosis*

One off-shipping case was reported in Hawaii in 1929-1930.

#### *Smallpox*

Smallpox has been reported in different places on the north coast and in the surrounding islands of Dutch New Guinea. An epidemic was suppressed by vaccination, and no cases have been observed since 1917. Towards the close of the last century there appear to have been serious outbreaks of smallpox in New Britain and the Admiralty Islands in New Guinea Territory.

In the Japanese Mandated Islands, smallpox is ported from the Palau, Marianas, Carolines, and Marshalls. It was introduced into the Palau in 1783 and into the Carolines in 1854. In 1856 it reached the Marianas. It was reported in the Marshalls in 1908-1909. Cases were reported on Guam in 1924-1925 and in undesignated places in the Mandate in the League of Nations reports for the Japanese Mandated Islands for 1926-1928.

Smallpox has invaded Tahiti more than once, and cases were reported there as recently as 1939. In the Marquesas in 1863 it reduced the population of Nukuhiva by half, and in 1866 it killed off a quarter of the inhabitants of that island and Uapou. On Easter Island, there was an epidemic during the 1860's, when the population was almost exterminated. Smallpox is reported also from the Gambiers.

McKinley states that there has been no smallpox in Hawaii since 1903. A case has been reported off-shipping in 1937. Immunization of the entire population was undertaken in 1942.

Smallpox is believed to be absent from the Solomon Islands, New Hebrides, New Caledonia, the Loyalty Islands, the Gilbert and Ellice Islands, Fiji, the Wallis and Horn Islands, and American Samoa.

#### *Trachoma (and Epitheliosis desquamativa conjunctivae)*

Trachoma is reported as endemic in New Guinea Territory and the Territory of Papua, but the incidence of the disease there is not high. It has become very common in the Japanese Mandated Islands. It is reported on Guam.

In the British Solomon Islands Protectorate, trachoma is most prevalent in the San Cristobal and Guadalcanal districts with 6 to 8% incidence among the natives. It is reported from the New Hebrides, Nauru, the Gilbert and Ellice Islands, Tonga, Western Samoa and the Cook Islands.

Trachoma is still present in Fiji, but the proportion it has to the total of eye diseases, which are common in some parts of the group, is unknown. In Hawaii over a thousand cases have been reported in a single year. However, the Territorial Bacteriologist says that at present only about one case per month is reported.

Leber and Prowazek separated from trachoma as a separate disease entity, epitheliosis desquamativa conjunctivae. It is a virus disease which they state occurs in New Guinea, Saipan in the

Marianas, New Zealand, Tonga and Samoa. Lambert refers to a "trachoma-like" condition in Fiji. Born observed on Yap in the Carolines a disease like follicular conjunctivitis which he believed might be trachoma; this may be related. On Guam there is among children a widespread chronic follicular conjunctivitis resembling trachoma but without its characteristic sequelae.

The identity with or separation from trachoma of these conditions awaits application of modern techniques.

#### *Trench fever*

A case of trench fever, a disease now believed to be caused by *Rickettsia*, has been reported from Levuka, Fiji. *Pediculus humanus* is reported there and elsewhere in the Pacific.

#### *Rickettsioses (Scrub typhus and Endemic or murine typhus)*

Scrub typhus (= mite fever or tsutsugamushi fever) is absent from Dutch New Guinea, according to DeRook, but the presence of possibly suitable mite vectors in that colony and the proximity of cases in the Mandated Territory in an area not separated by important natural barriers leaves the question in doubt.

In New Guinea Territory a similar exanthem was reported during the period of German administration at Rabaul and Kokopo, while authenticated cases are now recorded from Green River and the lower Sepik drainage, from Aitape, Wewak and Madang on the coast, Karkar Island, the Watut (Bulwa-Bulolo-Wau gold fields area) and Ramu River valleys and from the Kokopo-Bita Paka area on New Britain Island. With one exception (a Chinese girl), the disease has been in occidentals. 101 cases have been reported currently as treated at the U. S. Army 1st Evacuation Hospital, New Guinea. An outbreak in natives, possibly of the same disease, occurred near Kieta, Bougainville Island, in 1930.

Fatal cases among native hospital orderlies at Port Moresby, Papua, have been ascribed to scrub typhus and two non-fatal cases of that disease in Europeans working in the Laloki valley have been confirmed serologically. During the Buna-Gona campaign (along the northern coast of Papua Territory) the disease appeared among allied troops with a number of fatal cases. In this outbreak serological and histopathological diagnosis of scrub typhus was made.

Cases of typhus fever (*typhus exanthematicus*) were reported as acquired in Ponape (East Caroline Islands) during the German occupation.

Innes observed an outbreak suggesting typhus in the southern portion of Malaita, Solomon Islands Protectorate. Poleck identified eight cases of an exanthematous fever in Western Samoa as pseudo-typhoid of Schüffner (i.e. scrub typhus).

Endemic (murine) typhus fever, serologically similar to the Wilmington strain, is widespread and a major health problem in the Territory of Hawaii. Rats of Honolulu have been found to act as reservoirs of the rickettsia and fleas (experimentally) to serve as vectors. Approximately 80 cases, about two thirds of them in Honolulu, are reported yearly. The cases are sporadic and rarely involve more than one person in a household. A very few deaths have been recorded since the first reports of the disease (as Brill's disease, 1914) from the archipelago.

#### *Yellow fever (?)*

A case of yellow fever was reported to have been landed in Hawaii in 1911, and the native watchman stationed as guard over the patient "was taken sick at his home." The Report to the League of Nations on the Japanese Mandated Islands records a case of yellow fever treated at a South Seas Bureau Hospital in 1930. It seems more likely that acute infectious hepatitis or epidemic jaundice (*q. v.*) was involved.

*Aedes aegypti*, the vector responsible for extensive outbreaks of urban yellow fever elsewhere is widespread in the Pacific.

Although the Hawaiian cases were reported before the use of viscerotomy and the "protection tests," the diagnoses of yellow fever were made by experienced authorities, and the cases were recorded as yellow fever in the annual reports of the United States War Department and Public Health Service. So certain were the Hawaiian citizens and officials that the diagnoses were correct, that they spent over \$100,000 on immediate yellow fever preventive measures.

#### DISEASES CAUSED BY BACTERIA

##### *Anthrax*

There are reports of anthrax from the Japanese Mandated Islands, New Caledonia and Hawaii. In Hawaii about one case is reported every two years.

##### *Asiatic cholera*

It is reported that on at least one occasion, in 1913, the specific organism of Asiatic cholera was identified under conditions which pointed to the unrecognized existence of sporadic infection in German New Guinea.

In 1901 an epidemic of a disease similar to Asiatic cholera was reported in New Caledonia. Five cases of the disease were reported there at that time on a ship from Java. In 1903 two cases of Asiatic cholera were confirmed in an orphanage near Noumea.

McKinley reports that there has been no Asiatic cholera in Hawaii since 1890, but its existence there in 1895 is recorded.

##### *Bacillary dysentery*

In Dutch New Guinea, bacillary dysentery is occasionally prevalent in small epidemics on the north coast and nearby islands.

From an economic point of view, bacillary dysentery, with complications, is the most important disease in New Guinea Territory. It was violently epidemic there from 1885 to 1887 and has remained in a milder epidemic form. It is one of the principal causes of morbidity and death. The League of Nations Report for the Territory in 1936-1937 refers to the isolation of the Flexner dysentery bacillus, and to a strain allied to the Schmitz type and provisionally named *Bacillus rabaulensis*. Bacillary dysentery due to true Flexner infections predominates over those due to "Rabaul type bacillus." The disease is reported in the Territory specifically from the New Guinea mainland, New Britain, New Ireland, and Bougainville and Buka Islands. On New Britain, where it claims numerous victims, the annual incidence wave is usually at a peak in the dry weather between May and August. The Flexner type of dysentery was epidemic in 1928-1929 at Rabaul; Shiga was found in two cases there in 1926-1927, and Y strain was found in the majority of cases there in 1922-1923. The League reports for 1928-1929 record an epidemic of Flexner dysentery at Kavieng, New Ireland, where the disease is said to be endemic. The 1935-1936 report states that the disease is epidemic on Bougainville Island, and in the following year it was reported on the adjacent island, Buka.

Bacillary dysentery has previously been reported as an important problem in Papua. In 1926 there were still a few cases and at times small epidemics.



One early epidemic, data unspecified, is said to have "raged for thirteen months and depopulated great tracts of country entirely." Ultimately the disease became endemic, and it remains so today.

From 1899 to 1914 bacillary dysentery was listed in official German reports from Saipan in the Marianas, Yap and Ponape in the Carolines, and Jaluit in the Marshalls. However, after the islands came under the Japanese mandate, in the reports to the League of Nations, no separate entries are made for bacillary dysentery; it is merely included in the category "diseases of the digestive tract."

In Guam, bacillary dysentery was epidemic in 1924-1925.

Dysentery, mainly bacillary, is reported as responsible for the heavy death rate in the Solomon Islands. Outbreaks were reported there in 1936 and 1937, and in 1939 the disease was reported as endemic and widespread.

In 1921-1922, Davies stated that the New Hebrides appeared to be free of bacillary dysentery, but in the New Hebrides Annual Report for 1923, it is reported as "one of the main causes of death." The disease is reported in Noumea, New Caledonia.

An outbreak of bacillary dysentery among Chinese employees at the Pacific Phosphate Company on Nauru was reported as early as 1906-1907; it was probably of the Shiga type. The British Administrator of Nauru has recorded a number of cases since. In the Gilbert and Ellice Islands it is reported as prevalent and endemic.

A number of epidemics of bacillary dysentery have been reported from Fiji. The outbreaks of 1930 and 1931 were of the Shiga type, while in 1932 the Flexner type predominated. In 1936, Shiga, Flexner, Schmitz, and Sonne strains were all reported. It has been suggested that the bacillary type was introduced at the same time as measles, in 1875. It is now endemic there.

Bacillary dysentery occurs in yearly epidemics in American Samoa. In a 1930 report of its presence there, cultures were reported negative except in two instances in which the Flexner-Strong type was recovered. In Western Samoa it is reported as prevalent and epidemic.

In 1926, bacillary dysentery was reported as uncommon in the Cook Islands. In the Territory of Hawaii, Flexner, Sonne, Hiss and Strong dysentery are endemic, but Shiga is not known to be present.

### *Cerebrospinal meningitis, epidemic (epidemic cerebrospinal fever, meningococcal meningitis)*

Cerebrospinal meningitis occurs sporadically in New Guinea Territory and Papua. It is reported as a common cause of death in New Britain. It occurs in New Ireland also.

In the Palau Islands, infectious cerebrospinal meningitis has been reported among the Japanese. In Guam, cases are reported as recently as 1940-1941.

Cerebrospinal meningitis is not endemic in the British Solomon Islands Protectorate but it has been introduced and may occur in epidemic form.

An outbreak of cerebrospinal meningitis on board ship off Noumea, New Caledonia, was reported in 1921. Sixty-six coolies were infected; twenty-eight died. An epidemic of cerebrospinal fever was reported in the New Hebrides in 1921-1922, and cases of cerebrospinal meningitis were reported there in 1930 and 1932. A fatal case of meningococcal meningitis was reported on Nauru in 1935.

Davidson has described an epidemic of cerebrospinal meningitis which started in Fiji among emigrants from New Britain and New Ireland. There were 128 cases, of which 90 terminated fatally. Meningococcal meningitis and cerebrospinal meningitis, usually considered synonymous with epidemic cerebrospinal fever, were reported in Fiji in the period 1935-1937. Cerebrospinal meningitis is reported from both American and Western Samoa.

Meningococcal meningitis was reported by the Hawaii Board of Health at least as early as 1882-1883. An outbreak of 203 cases of the disease with 79 deaths occurred during 1929. Cerebrospinal fever fatalities are recorded each year from 1931-1938, none in 1938-1939, but again each year to 1942-1943, when 37 cases were reported from the Territory.

### *Chancroid*

Soft chancre has been reported from New Britain, New Ireland, the Caroline Islands, the Marshalls, Nauru, the British Solomon Islands Protectorate, Fiji, Western Samoa, and Tahiti. Hawaii had an epidemic of 25 cases during the summer of 1942.

### *Diphtheria*

Lambert reports that diphtheria occurs in a mild form in many of the islands of the South Pacific, but it rarely becomes malignant.

The infection is reported from Dutch New Guinea, New Guinea Territory, the Territory of Papua, New Caledonia, the New Hebrides, the Japanese Mandated Islands and Guam, the Gilbert Islands, Fiji, Tonga, and Hawaii. Although it is not known to exist in the Solomons, cases resembling it have been observed there.

On New Britain in New Guinea Territory, there was an abortive outbreak in 1935, when a strain indistinguishable from *Corynebacterium diphtheriae* was isolated. It proved avirulent in the laboratory. In the Territory of Papua a few cases of diphtheria have occurred, always during the coldest parts of the year.

In 1928 there were 35 cases of diphtheria in New Caledonia on board the *Cassiopee*. Three cases were reported on the island in 1937.

In 1932, Placidi reported that diphtheria was unknown in the New Hebrides; three cases, however, had been reported there in 1928. The disease exists in the Gilbert Islands. In Fiji, it has been reported that clinical cases occur only sporadically and virulence is low. However the Klebs-Loeffler bacillus has been isolated, carriers are not uncommon, and there are grounds for believing that a more virulent type of diphtheria is on the increase.

At Nukualofa, the capitol of Tongatabu, Tonga Islands, two cases of diphtheria were isolated in 1932. According to the Resident Commissioner of the Cook Islands, diphtheria is not known in that group.

In Hawaii, records of diphtheria are given in the reports of the Board of Health as early as 1901. The disease is reported regularly throughout the Territory. Most cases are of the intermedius strain, the mitis strain being occasional and the gravis strain rare if not absent.

### *Erysipelas*

In the Japanese Mandated Islands, cases of erysipelas are reported in the Palaus, the Marianas, the Carolines, and the Marshalls. The Reports to the League of Nations contain references to cases among both Japanese and natives. It is listed from Guam as recently as 1940-1941.

Cases of erysipelas are noted annually in the British Solomons *Blue Book*. In the Nauru *Reports of the Administrator*, one case is noted each year from 1922-1926. The disease is reported in the Gilbert Islands. In Fiji, from 1 to 3 cases were listed annually from 1932 to 1938.

Erysipelas is reported from both Western and American Samoa, and isolated cases have been recorded in the Cook Islands. The Hawaii Board of Health reports the disease there as early as 1888-1889. In 1937-1938 there were 67 cases, but since then the number has fallen to about two a month.

### *Gonorrhoea*

In Dutch New Guinea, gonorrhoea is said to be "slightly prevalent" in places where Malaysians have settled. In New Guinea Territory it is endemic wherever indentured laborers have come in contact with the coastal tribes. As early as 1903-1904, it was reported as the most common venereal disease of the natives of north-east New Guinea and of the residents of Kokopo, New Britain. The League of Nations reports on New Guinea Territory mention a village in the Talasea District in which 80% of the adults were found to be infected. Kersten records gonorrhoea as very common in New Britain. In 1905 it was reported to be a "virtual plague" at Kavieng, New Ireland, and spreading all over the islands. The Reports to the League of Nations on New Guinea Territory in 1925-1926 indicate that the northern part of New Ireland is heavily infected, although the disease is not common in Namatanai, about 135 miles south-east of Kavieng. The same report states that gonorrhoea is very prevalent on Bougainville, rare on Buka Island, and of 6.3% incidence on Manus Island in the Admiralties. In 1933 it was stated on the strength of earlier observations that infectious venereal diseases did not occur in the St. Matthias group, but they may do so now.

Strong states that in the Territory of Papua, gonorrhoea is especially troublesome in particular districts, though on the whole "less prevalent than in a civilized European country." It is reported on both Samarai Island and the Papuan mainland.

In the Japanese Mandated Islands, gonorrhoea is reported from the Palaus, Marianas, Carolines, and Marshalls. The 1932 Report to the League of Nations stated that 33% of the natives on Yap in the Carolines were suffering from this infection. It is also reported from Guam.

Cilento states that throughout Melanesia gonorrhoea is prevalent and widespread in areas where laborers have returned from indenture, a view concurred in by Strong. Cilento reports a marked resistance of the disease to treatment.

In the British Solomon Islands Protectorate,

gonorrhea is reported as fairly common in the ports, and on certain parts of the coast, particularly southern Guadalcanal. In the New Hebrides, Lambert reports it as becoming increasingly common, while Placidi regards it as rare there. Kermorgant reported gonorrhea in imported Japanese miners in New Caledonia and feared that it would spread. Most of the 62 hospitalizations for venereal disease in New Caledonia in 1929 were due to gonorrhea. Fifty per cent of the Javanese in New Caledonia are reported to be infected. Most of the males in the Loyalty Islands have gonorrhea. In the Gilbert and Ellice Islands there is evidence that the disease is gaining. Cases are reported from time to time at the British Government Hospital and the Phosphate Company hospital at Nauru. In Fiji, gonorrhea is reported as common. It is reported also from Tonga.

Gonorrhea has been reported as increasing in Western Samoa. Buxton says that although it is sometimes maintained that Samoans are immune to gonorrhea, of five urethral smears in which *Gonococcus* was found, four were from pure-bred Samoan males, and he suggests that more natives are infected than is realized. In American Samoa, gonorrhea has been reported in both the natives and the naval personnel.

Cases are reported in the Niue Resident Commissioner's reports, 150 in 1932 alone. Gonorrhea is said to be very common but not always reported in the Wallis Islands. In both Wallis and Horn Islands, it is rarely treated in dispensaries. In the Cook Islands, Lambert reports it as uncommon outside of Rarotonga.

Gonorrhea is reported as increasing in Tahiti. In the Marquesas, as Buisson points out, it is apt to disappear for periods only to break out in an epidemic after a fête or the arrival of a ship. In the Territory of Hawaii, nearly one hundred cases, most of them from the Island of Oahu, are reported monthly to the Board of Health.

### *Impetigo*

Impetigo has been reported as common in New Guinea Territory. It is said to be one of the chief diseases encountered on Guam. In the British Solomon Islands Protectorate it is reported as widespread among the natives. On Nauru the Administrator lists from two to fourteen cases annually.

Impetigo is reported as common in the Gilbert and Ellice Islands as well as on Fiji, in Western

Samoa, and on Niue. It has been reported in the Wallis and Horn Islands. In the Cook Islands it is said to be relatively rare.

Buisson reports impetigo in the Marquesas. In the Hawaiian archipelago it is mainly a disease of children. During the fiscal year 1942-1943, 261 cases were reported from the Territory.

### *Leprosy*

The presence of leprosy in the Pacific Islands has long been known. The dates of introduction are of importance, for in the regions infected earliest, the disease has passed its maximum incidence and severity. In relatively recently infected regions, the disease is likely to be very severe.

Leprosy is reported throughout Dutch New Guinea to a light extent, except in one region on the north coast.

In New Guinea Territory it is reported prevalent in all districts. Cases are listed specifically from north-east New Guinea, New Britain, New Ireland, New Hanover, the St. Matthias Islands, the Admiralty Islands, and Bougainville and Buka Islands. On New Ireland, leprosy was noted as early as 1837; 32 cases were reported there in 1928, and by 1935 there were 547. On New Britain, the first case was reported in 1906, infection having been attributed to contact with the Chinese, and by 1937, 500 cases had been confirmed bacteriologically.

Leprosy is said to be of recent introduction in Papua. It is reported as prevalent in all districts, and cases are recorded specifically from south-east New Guinea and the D'Entrecasteaux, Louisiade, and Trobriand groups.

In 1935 it was stated that there had been a large number of lepers scattered all over the Japanese Mandated Islands from early times. The disease was specifically reported from the Palaus, the Marianas, the Carolines, and the Marshalls. Leprosy was not considered common on Guam during the United States Administration, 1898 to 1941, though 113 cases were known there in 1907.

In the British Solomon Islands Protectorate, leprosy is widespread, and new cases are being found continually. Incidence is said to be heavier in the bush than in the coastal areas. The disease is reported especially on Malaita, N'Gela, (Florida), Guadalcanal, Ysabel, Gizo and the eastern Solomons, in order of severity, though it is reported from all districts.

Leprosy was brought to New Caledonia by the

Chinese and it is at present the most serious disease there.

Leprosy was introduced into the Isle of Pines by natives from New Caledonia after the insurrection of 1878. Infected and suspected cases involved 5.8% of the island population in 1924. The disease appeared in the Loyalty Islands about the same time as in New Caledonia.

In the New Hebrides no census of leprosy has ever been taken. Pentecost Island in this group was called Leper's Island on the early charts, confusion apparently having arisen from the inability to distinguish between this disease and ulcerative tertiary yaws. Leprosy was brought to the New Hebrides from Queensland and New Caledonia by repatriated Kanakas, but it is not sufficiently common to be a serious social danger there.

The history of leprosy on Nauru appears to have been brief. It was introduced there between 1910 and 1914, but there appears to have been no visible evidence of the infection among the population in general until 1920, when four cases were reported as suspected. An epidemic of influenza in 1921 affected the entire population, and the resulting lowered resistance may have been responsible for some of the ten cases of leprosy discovered in 1921 and the 193 cases (30% of the population) segregated by 1924. Since that time there has been a gradual decline in morbidity. No new cases were reported in 1938.

Leprosy occurs on all of the islands in the Gilberts, but the disease appears to be absent from the Ellice group. Cases are reported from Rotuma.

The island of Makogai in Fiji is devoted to the treatment of leprosy. Under a cooperative scheme its facilities have been extended to New Zealand, Rotuma, Tonga, Samoa, Niue, and the Cook islands.

Leprosy is reported in the Wallis and Horn Islands, and in Tonga. In 1892 Davies offered the first medical evidence of the disease in Samoa. It must, however, have been present there for some time before this. Cases are reported in Niue. Tokelau was without leprosy in 1927.

Tongareva or Penrhyn in the Cook Islands is described as a "hotbed of leprosy"; the disease was introduced there in 1885 from Hawaii. The infection is reported as prevalent in the northern Cook Islands, especially on Tongareva. In the south, Palmerston Atoll, Aitutaki, Rarotonga, and Atiu have had cases.

Leprosy is said to have been in Tahiti since ancient times. Some maintained that it was introduced by the Chinese, but missionaries were of the opinion that the disease existed there earlier.

In the Marquesas also, leprosy is believed to have existed before the arrival of the Chinese. Clavel, who went there in 1881-1882, maintained that at that time leprosy was more prevalent there than in any other country in the world, and he maintained that the Marquesans had known the disease from time immemorial. In the Tuamotus and Gambiers it has long been disastrous. In 1935 Massal stated that the Tuamotus threatened to become vast leprosaria unless the rapid increase of the disease was checked. At that time 56 of the 340 inhabitants of "Rheo," and 23 of the 180 inhabitants of Puka-Ruha were reported to be infected.

Leprosy is reported also from Rapa and the Tubuai-Australis, and from Easter Island.

Leprosy was introduced into the Hawaiian group about 1850, and segregation has been carried on there since 1864. Since 1921 about three cases have been reported monthly from the Territory.

#### *Paratyphoid fever*

Paratyphoid fever is reported as uncommon and mild in New Guinea Territory and Papua. The reports to the League of Nations record it from the Japanese Mandated Islands. Some cases of paratyphoid occurred on Truk and Ponape in the Carolines. A violent outbreak of typhoid and paratyphoid on Tinian Island in the Marianas is reported to have infected over 300 victims between 1929 and 1931. According to the 1926 Annual Report of the Governor of Guam, no cases of paratyphoid were diagnosed there, but the disease is recorded in the same series of reports for 1923-1924 and for 1931-1932.

McKinley reports that typhoid and paratyphoid fevers are not present in the British Solomon Islands Protectorate. Horack, however, reports "typhoid-paratyphoid" as endemic, and sporadically assuming epidemic proportions there. Paratyphoid is reported from New Caledonia, and the New Hebrides, Fiji, American and Western Samoa, and the Cook Islands. The Pitcairn Island *Medical Report* for 1937 notes a case on a passing freight steamer.

Paratyphoid has been listed separately from typhoid in the Hawaii Board of Health Reports since 1939-1940. A mass immunization program

was conducted throughout the Territory in 1942 against typhoid and paratyphoid fevers. Only one case (paratyphoid A) of paratyphoid fever has been reported since the mass immunization, (up to September 1943).

### Plague

Yap in the Carolines had an outbreak of 143 cases of plague in 1910-1911.

Plague was introduced into Noumea, New Caledonia, in 1899. The epidemic raged into March, 1900, and cases were observed in 1901. In 1903 it appeared in the northern part of the island, and in 1905 and 1906 at Noumea again. In 1912 it again broke out in serious proportions, and by 1917 it was regarded as endemic. In February, 1941, there was an outbreak of bubonic plague at the Port of Goro, followed by two fatal cases of pneumonic plague in November of the same year. The source of infection seemed to be native brush coconut tree rats. An additional case of pneumonic plague appeared in New Caledonia in September, 1942.

The first reported cases of plague in the Hawaiian Islands occurred in Honolulu in December, 1899. The disease appeared on Maui and Hawaii in 1900, and in Kauai in 1901. Infected rodents and infected fleas are endemic along the Hamakua coast on the island of Hawaii and in the Makawao area of the island of Maui. After a lapse of two years with no human cases, five fatal cases of human bubonic plague have occurred on the Hamakua coast in 1943.

*Xenopsylla cheopis* is specifically reported from New Guinea, Samoa, Hawaii and the Marquesas and may be expected to occur elsewhere. In the Japanese Mandated Islands, according to Esaki, fleas are "very rarely met with, if there are any."

"Pneumonias" (see also "Oahu fever")

The pneumonias are important causes of morbidity and mortality in New Guinea Territory. They are endemic and periodically epidemic with high mortality there. They are particularly prevalent at the beginning and end of the rainy season. Inadequate diet is believed to be an important factor in predisposing the natives to these diseases, which in 1927-1928 accounted for about 14% of the total mortality. The pneumonias are reported within the Territory from New Britain, New Ireland, St. Matthias Island, the Admiralty Islands, and Bougainville and Buka Islands. In 1935-1936

they were reported as the principal cause of death at Gasmata, New Britain. In 1939, 311 of 1,283 autopsies at Rabaul, New Britain, showed cause of death as pneumonias.

The pneumonias are not uncommon in epidemic form in the villages of Papua. They are reported within the Territory of Papua from the Trobriand Islands, the D'Entrecasteaux group and Samarai Island.

Among the Japanese Mandated Islands, the pneumonias are reported from the Palaus, Marianas, Carolines, and Marshalls, and they take first place in morbidity statistics in the East Carolines. They are reported on Guam.

The pneumonias have been reported to be the principal cause of death of indentured laborers in the Solomon Islands. They were particularly prevalent in 1936 and 1937. They are reported as among the most fatal diseases of the natives of New Caledonia, where they are very common. They are reported on all the islands of the Loyalty group. In the New Hebrides they are reported as among the most fatal diseases.

The pneumonias are reported also from Nauru and the Gilbert and Ellice Islands. They are common in Fiji, and were said to be very prevalent there in 1928. They are reported also from Tonga and Western Samoa. In 1941 the pneumonias were among the leading causes of death in American Samoa. They are reported from Niue, the Cook Islands, the Society Islands, and Pitcairn.

Lobar pneumonia is recorded in the Hawaiian Board of Health reports from 1882-1883 to date. In 1892, Davidson stated that pneumonias were of frequent occurrence in the Hawaiian Islands but are only moderately fatal there. McKinley states that they constitute an important public health problem. In recent years, an average of 350 cases has been reported though there were only 188 cases in 1942-1943.

### *Pyomyositis, tropical (Myositis Purulenta Tropica, Tropical Myositis)*

Pyomyositis is reported from New Guinea Territory, the Territory of Papua, the British Solomon Islands Protectorate, the New Hebrides, Fiji, Tonga, the Gilbert Islands, Samoa, Niue, and the Cook Islands. Manson-Bahr reports that the organisms concerned have been "*Staphylococcus aureus* and *S. albus*, and occasionally *Streptococcus pyogenes*."

*Rat-bite Fever*

A case of rat-bite fever was reported in New Caledonia in 1925, at which time the microscopic diagnosis of *Spirillum minus* was first established there. A case was noted in Fiji in 1925. The disease has also been reported throughout the Territory of Hawaii. A case occurred in 1942 at Pahela on the island of Hawaii.

Rat-bite fever was reported in a patient admitted to Rabaul Hospital, New Britain, but the accuracy of the diagnosis is said to be invalidated by the account of the organism.

It may be anticipated that further cases of rat-bite fever, when carefully sought for, will be diagnosed elsewhere in the Pacific where rats prevail and live in close association with man.

*Scarlet fever*

Scarlet fever is reported from Guam, New Caledonia, Nauru, Fiji, and Hawaii. It is said to be absent from New Guinea Territory, Papua, the British Solomon Islands Protectorate, the New Hebrides, the Cook Islands, and Pitcairn. McKinley reported it absent from Guam but overlooked the cases reported there in 1924.

McKinley states that scarlet fever is present in Hawaii but is not an important health problem. In a light form it is present throughout all the islands of the Territory; it is reported regularly, but results in few deaths.

*Septic sore throat* (see also *Staphylococcus* and *Streptococcus* infections.)

Septic sore throat is listed by the Hawaii Board of Health as a cause of morbidity in all islands of the Territory. "Suva throat," from which Fijian school children suffer severely, is associated with streptococci.

*Staphylococcus* and *Streptococcus* infections (see also *Pyomyositis* and *Septic sore throat*.)

Staphylococcal infection is reported as relatively common in Fiji, and mixed staphylococcus-streptococcus infections are reported on Tahiti. *Streptococcus viridans* is also reported in Fiji. A bacterial endocarditis caused by the same organism occurs in Hawaii but is not reportable.

*Tetanus*

Tetanus is reported occurring in the Territory of New Guinea, Papua, the Palaus, the Marianas, the British Solomon Islands Protectorate, and the

Santa Cruz Islands. It is reported as causing the deaths of many children in the New Hebrides and New Caledonia. Vaccination of all Tonkinese workers in the New Hebrides was reported in 1936. In the Fiji Islands, the infection is reported constantly.

Tetanus is said to be prevalent from time to time in some of the Ellice Islands, especially Vaitupu and Nukufetau. It is listed from Tonga and from both Western and American Samoa. Tetanus is said to occur in the Cook Islands from time to time. It is recorded specifically from Mauke Island, the easternmost of the Cook group. Tetanus is widespread on Tahiti and was reported from Pitcairn as early as 1838. It has been reported in Hawaii since 1882-1883. Thirty cases, eight fatal, were reported in 1941-1942.

*Tonsillitis, acute*

Tonsillitis is reported from Port Moresby in the Central Division of the Territory of Papua, and from the Samarai and Woodlark groups. It is reported in the British Solomon Islands Protectorate and on Nauru. Cases have been hospitalized in the Gilbert and Ellice Islands. It is reported from Fiji, American Samoa, Niue, and the Cook Islands. It was reported by the Hawaii Board of Health as early as 1894, and still exists there.

*Tuberculosis*

Tuberculosis is spread evenly through the most settled portions of New Guinea Territory and is one of the principal causes of death and morbidity there and in Papua. In New Guinea Territory, it is reported on the mainland and from New Britain, New Ireland, the Admiralties, Bougainville and Buka Islands, and the mainland. In the Territory of Papua it is reported from Samarai Island and the Trobriand Islands.

Tuberculosis is prevalent in the Palaus, Carolines, Marianas, and Marshalls. It is the leading single cause of death on Guam.

Tuberculosis is endemic and one of the most frequent causes of death in the British Solomon Islands Protectorate, where it is very prevalent. About 75% of the total population is reported as tuberculin positive. As early as 1862, it was reported to be the scourge of New Caledonia. Almost all the Kanaka tribes there are infected. There is a high mortality. A quarter of the total population has been reported infected. In the

Loyalty Islands, tuberculosis is very active, killing off entire families. In the New Hebrides it is widespread and endemic and the most serious disease of the natives. It is reported on Nauru.

In the Gilbert and Ellice Islands, tuberculosis has long been prevalent. It is worse in the Ellice than in the Gilbert group and is described as the most fatal disease there. It is one of the most prevalent communicable diseases in Fiji. It is reported from Wallis and Horn Islands. In Tonga it is the commonest cause of death.

In 1941 tuberculosis was reported as one of the leading causes of death in American Samoa. On Niue it has increased from being not very prevalent in 1913-1914 to the "most serious health problem" in 1934-1935. In the Tokelau group it is less prevalent. In both groups of the Cook Islands, tuberculosis is the principal disease.

In Tahiti, Davidson reported tuberculosis as responsible for 25% of deaths. It is common in the Tuamotus. In the Marquesas it is believed to be one of the principal causes of depopulation. It is the most feared disease there, as it acts with great rapidity. It is the dominant disease on the Gambier Islands. It is listed from Pitcairn, and in the late 1860's it was very prevalent on Easter Island. In Hawaii, tuberculosis rates are higher than the averages of the United States mainland. Disabilities and deaths are largely concentrated in non-Caucasians of the highly congested areas.

### *Typhoid fever*

Typhoid fever has been reported recently as endemic throughout all regions of New Guinea Territory and Papua. Hitherto it had been reported as uncommon and not severe there. In New Guinea Territory it is reported on New Britain, the Duke of York Islands, and Bougainville and Buka Islands. In the Territory of Papua cases are reported on the mainland and on Samarai Islands.

Typhoid fever is present in the Japanese Mandated Islands. On Guam it has not been important in the white population since the improvement of the water supply in 1900, but it remains endemic in the native population.

In 1935, it was stated that no case of typhoid fever had been seen or reported anywhere in the British Solomon Islands Protectorate at any time. Horack, however, has recently reported typhoid-paratyphoid there.

Typhoid fever became established in New Cal-

donia shortly after the French occupation of 1853. The disease is now endemic at Noumea. In 1927, 192 cases were reported, and water purification was begun in 1928. The New Hebrides are free of the infection.

Typhoid fever is reported from the Gilbert and Ellice Islands and on Nauru. In the Fiji Islands it is endemic, but it has not been epidemic in Suva since an outbreak in 1925. Cases are scattered and confined mostly to low-lying areas. A case of typhoid on Ono-I-Lau in the Fiji group was verified in August 1943. It is rare on Wallis and Horn Islands, and endemic in Tonga. It is reported in Western and American Samoa but does not seem to be very common. Several outbreaks of typhoid fever are reported on Niue, but the most recent was in 1936. In the Cook Islands, outbreaks of typhoid occur periodically on Rarotonga and Mangaia, and a small epidemic was reported on Atiu in 1937-1938. The disease was reported in the Tubuai-Australs in 1931. It has been reported as endemic in certain valleys of Tahiti and Moorea in the Society Islands. It is endemic in the Marquesas. In the Hawaiian Islands, typhoid fever is endemic, but known carriers are registered. Mass immunization against typhoid and paratyphoid fevers in 1942 was followed by only nine cases of typhoid fever in the entire fiscal year, 1942-1943.

### *Undulant fever*

Undulant fever is reported from the Marshalls, the New Hebrides, Fiji, and Hawaii. In Hawaii there are noted on the average less than six cases yearly. In the New Hebrides, cow's milk was proved to be the source of infection in at least one case.

### *Whooping cough*

Whooping cough is reported as occurring sporadically among both European and native populations in New Guinea Territory and Papua. In New Guinea Territory it is reported on the mainland, and from New Britain, the Duke of York Islands, New Ireland, the Admiralty Islands, Bougainville, and Buka. In the Territory of Papua, whooping cough is endemic.

Whooping cough appears to be common in the Japanese Mandated Islands. It was epidemic in the Marianas by 1885, and it was prevalent in the Carolines by 1899. It is common on Guam.

Whooping cough occurs sporadically in the



British Solomon Islands Protectorate. Epidemics are reported from New Caledonia and the New Hebrides. A particularly severe outbreak of whooping cough was reported in the northern islands of the Ellice group in 1913-1914.

In 1883 there was a serious epidemic of whooping cough in the Fiji Islands with 3,000 victims among the natives. Whooping cough has been prevalent there in recent years, the disease being at its worst in the drier months.

An epidemic has been reported from Wallis and Horn Islands. Whooping cough first made its appearance in Western Samoa in the 1840's. An epidemic there in 1936-1937 spread in the latter year to American Samoa, where it had also been known earlier. A mild epidemic was recorded in Rarotonga in the Cook Islands in 1929-1930. Tahiti had an epidemic of whooping cough in 1912. The disease was also reported from Pitcairn. It occurred in the Hawaiian Islands as early as the end of the 1930's and has been recurrent there since 1855. It is probably present on all islands of the group. 1,531 cases were reported in 1942-1943.

#### DISEASES CAUSED BY FUNGI

Knowledge of diseases due to fungi occurring in the Pacific Islands is fragmentary and indefinite. The specialized techniques of mycology do not appear to have been used in this area. Symptomatology of these diseases, when given at all, is vague, and in only a few cases has there been any attempt to classify the species of fungus concerned.

#### *The Superficial Mycoses*

There are abundant references to "ringworm" in the reports of travellers in the Pacific Islands. Accounts by physicians often give no more definite designations. Some accounts give the extent of the involvement of the fungi, and it is clear that *Tinea capitis* (ringworm of the head) and *Tinea barbae* (ringworm of the beard) do occur. More often, however, the descriptions and illustrations indicate *Tinea glabrosa* (ringworm of the smooth skin).

Ringworm is particularly common among the natives of New Guinea Territory and Papua. Hamlin found more in the coastal regions than inland. In New Guinea Territory it is reported on the mainland and in New Britain, New Ireland, and the Admiralties. In the Territory of Papua it is reported on the mainland and in the D'Entre-

casteaux and Woodlark groups. In the northern D'Entrecasteaux nearly all of the natives are infected.

Ringworm occurs in the Japanese Mandated Islands and Guam.

In the British Solomon Islands Protectorate, approximately 8% of the population is infected with ringworm. Known as "buckra" by the planters it is one of the diseases diminishing efficiency of indentured laborers and is a cause of rejection by recruiters. It is endemo-epidemic in the New Hebrides, and mild though occasionally epidemic in the Gilbert and Ellice Islands. "*Tinea*" is reported from Nauru.

In Fiji ringworm affects a large percentage of the population. In Western Samoa, it is reported as common. "*Tinea*" was noted in Samoa for the first time in 1870. On Niue numerous cases were reported in 1940 and 1941. On Tokelau ringworm is very prevalent and in the Cook Islands it is common. In the Cook Islands ringworm is prevalent in Puka Puka (Danger Island). It was reported from Pitcairn in 1937.

The following clinical types of ringworm infection are reported from the Pacific Islands area:

#### *Tinea alba*

*Tinea alba* is very common in Samoa and Tokelau. It is prevalent throughout the Territory of Hawaii.

#### *Tinea albigena*

*Tinea albigena* occurs in New Guinea, and is reported from Palau.

#### *Tinea barbae*

Sycosis is reported as common in the British Solomon Islands Protectorate. In Hawaii *Tinea barbae* is prevalent throughout the group.

#### *Tinea circinata*

*Tinea circinata*, the classic form of ringworm (in some cases contrasted with *Tinea versicolor* or *Tinea imbricata*) is reported as widespread in the neighborhood of Rabaul, New Britain. It occurs in the Gilbert and Ellice Islands, and is reported as common on Niue. It is very common on Tokelau. In the Japanese Mandated Islands it is reported from the Palau, Caroline and Marshall groups. It is prevalent throughout the Hawaiian group.



*Tinea corporis tropicalis*

*Tinea corporis tropicalis* is very prevalent in the Tokelau group.

*Tinea cruris (Dhobie's itch)*

*Tinea cruris* is reported as very troublesome to Europeans in New Guinea Territory and Papua. It is found in the British Solomon Islands Protectorate and the New Hebrides, and is reported from Fiji, from American Samoa, and from Hawaii, where it is prevalent throughout the group. "*Eczema marginatum*," a synonym of *Tinea cruris*, is reported from the Palau group.

*Tinea imbricata (Tokelau Ringworm)*

*Tinea imbricata* is particularly common among the natives of New Guinea and Papua. In New Guinea Territory it is reported on the mainland and from New Britain and the Admiralty Islands. In the Territory of Papua it is reported on the mainland and on Samarai Island. Captain William Dampier found it in the Marianas in the seventeenth century. Guppy reported it in the Solomons in 1887. Recently it has been reported as one of the commonest skin diseases there. In New Caledonia it is found among new arrivals from the New Hebrides.

In the New Hebrides, *Tinea imbricata* is said to be found only in the northern islands and the Banks Islands. It occurs in the Loyalty Islands and was first found in the Gilbert Islands in 1841. In Fiji the incidence is high in certain districts. It is reported from the Wallis and Horn Islands and was described from the Gilbert Islands in 1844. It is reported from both Western and American Samoa. In 1929-1930 it was rare on Niue. On Tokelau, from which *Tinea imbricata* takes its common name, it is not prevalent but children are said to suffer from the infection. It is not as prevalent in the Cook Islands as in most Pacific Island groups. It is said to have been introduced there from the Gilberts. *Tinea imbricata* is found in Tahiti where it was introduced in 1864 by the natives of the Gilbert Islands imported for work on the plantations. It is said that the Tumaotus are not infected. In the Japanese Mandated Islands it is reported from the Palaus, Marianas, Carolines, and Marshalls. McKinley reports that it is not present in Hawaii.

*Tinea nigra (Pityriasis nigra)*

*Tinea nigra* is reported as present in American Samoa, of doubtful occurrence in Fiji and Gua,m

and as absent from the Solomon and Hawaiian Islands.

*Tinea versicolor (Pityriasis versicolor)*

*Tinea versicolor* is reported as very common in Oceania, where it frequently accompanies *Tinea imbricata*. "*Pityriasis*" is common in the Solomon Islands, and numerous cases of *Tinea versicolor* have been reported from natives and whites on Nauru. It is reported as present, but not common, in the Gilbert and Ellice Islands. It is reported on Fiji and American Samoa, and is common on Niue. It is reported in the Wallis and Horn Islands and in the Marquesas. Many cases have been reported from Yap in the Carolines, but its occurrence on Guam is considered doubtful by McKinley. It is reported as common on Jaluit in the Marshalls and as prevalent throughout the Hawaiian archipelago.

*Dermatophytosis*

*Tinea interdigitalis* is reported from the British Solomon Islands Protectorate. Dermatophytosis has been common among troops in the Solomons and the New Hebrides causing many sick days. The same species of fungus as is present on the feet has been in some cases responsible also for otomycosis in these men. *Epidermophyton interdigitale* is reported as fairly common in Fiji among Europeans and half-castes. It is endemic in Hawaii.

*Erythrasma*

This brownish red dermatomycosis is reported from Nauru, and from Jaluit in the Marshalls. It occurs in the Wallis and Horn Islands. It is present in the Territory of Hawaii.

*Moniliasis (Soor)*

A single case of "soor" was reported on Yap in the Carolines in 1906-1907. Moniliasis is found in the Territory of Hawaii.

## THE DEEP MYCOSES

*Actinomyces*

Cases of actinomyces are reported from Fiji and Tonga. Human cases are very rare in Fiji. However, actinomycosis in a bullock was demonstrated quite recently in the Pathological Laboratory, Suva, Fiji, in 1943. In Hawaii, the disease was reported in six of seven years preceding 1941-

1942. Cases of "lumpy jaw" appear in local cattle.

#### *Blastomycosis*

A fatal case of blastomycosis was reported from Papua in 1924-1925.

#### *Mycetoma (Madura foot)*

In 1928-1929 a native of New Britain was reported to have had for many years a foot that was swollen and discharging bodies similar to those formed by *Discomyces madurac*, the causative organism of a white-grained type of Madura foot. A case of Madura foot was reported in a native of Yap in the Carolines in 1900-1910. Two cases of the white variety of mycetoma were reported from American Samoa in 1907. In 1935 McKinley stated that mycetoma was absent from Hawaii, but a case was described from there in 1915, and another was reported in Honolulu in 1942.

#### *Sporotrichosis*

A case of pulmonary sporotrichosis was reported as treated at the British Phosphate Company Hospital at Nauru in 1926. In Hawaii in the last three years there has been reported approximately one case per year. These cases have been culturally as well as clinically positive.

### DISEASES CAUSED BY SPIROCHAETES

#### *Leptospirosis, and Seven-Day Fever*

In the Pacific, distinction between leptospiral jaundice and acute infectious hepatitis has rarely been made on the basis of serology or of the demonstration of *Leptospira*. In the absence of such identification, all reports of "icterus" must be considered indefinite.

The reports to the League of Nations on the Japanese Mandated Islands list two Japanese cases of "Wile's disease" at a South Seas Bureau Hospital in 1929 and a death from catarrhal icterus the following year. Cases of "catarrhal icterus" have been reported in the Marshalls.

Hepatitis has been reported from New Caledonia. "Catarrhal jaundice" has been recorded from Funafuti in the Ellice Islands. Cases of "acute hepatitis," hepatitis, jaundice, catarrhal jaundice, and "yellow atrophy" are reported from Fiji.

Since 1935 the Hospital in Apia, Western Samoa, has recorded cases of catarrhal and malignant jaundice. Dr. K. F. Meyer tested in 1940 the

blood sera from six jaundice cases there with every known strain of *Leptospira* with negative results. The kidney of one fatal case showed no *Leptospira*. These findings suggest that the "malignant jaundice" of Samoa is not caused by *Leptospira*. According to Dr. K. F. Meyer the "high mortality of 25% within a few days after onset suggest another infective agent for the malignant jaundice than the *Leptospira*. Some of the cases of jaundice may be caused by *Leptospira*. Infective hepatitis, also known as epidemic jaundice, or some new type of infection of unknown etiology is suspected."

Deaths from Weil's disease have been reported to the Hawaii Board of Health since 1907. Recently *Leptospira* has been demonstrated serologically in the Honolulu area, and antibodies of *Leptospira* have been found in the serum of human beings there. Since 1936, cases of leptospirosis have been found on Hawaii, Kauai, Lanai, Maui, and Oahu.

In 1942-1943 following the administration of yellow fever vaccine several thousand cases of hepatitis were reported in Hawaii. Other types of infectious hepatitis exist in the Territory, but these are not reportable. Sixty cases of acute infectious hepatitis which were not stated to be post-vaccinal were reported as admitted to a Honolulu hospital in one year.

Seven-day fever, due to *Leptospira hebdomadis*, was reported in New Guinea in 1939-1940.

#### *Relapsing fever*

*Spirochaeta recurrentis* (or *S. novae caledoniae*) is reported from New Caledonia, and it has been suggested that relapsing fever is not a rare disease there. It is not known whether it is tick or louse-borne, and if it is of local origin or imported.

#### *Yaws-Syphilis Complex*

Yaws is known to have been widely distributed in many of the Pacific islands at the time of their discovery by white explorers.

In Dutch New Guinea yaws is very prevalent, particularly on the north coast and in the adjacent islands. In the Territory of New Guinea and Papua, yaws is the commonest condition met with and is one of the principal causes of morbidity there. Cases are reported from the mainland, New Britain, the Duke of York Islands, New Ireland, and the Admiralty Islands.

In some districts of Papua almost everyone has yaws "infection latent." It is believed to have

been introduced there from Polynesia in the first half of the last century, but has made little progress inland. In the northern D'Entrecasteaux babies are often covered with yaws.

Yaws is reported from the Palaus, Carolines, Marianas, and Marshalls. It is the chief disease on Guam, where it is said to be endemic.

Yaws is endemic in the Solomons, where it is said to be universal among the natives. Lambert found it on both Rennel and Bellona. The infection rate is reported as 60% on Malaita and 65% on Makira; in "salt water natives" the incidence is higher than in "bush natives"—up to 90%. It causes great disability there, especially among the children.

New Caledonia is infected. Yaws is very widespread in the Loyalty Islands, where almost everyone gets it at one time or another. It is reported on Lifu in disquieting proportions, and it also occurs on Mare and Ouvéa. It is endemepidemic and exceedingly common in the New Hebrides.

The early issues of the *Medizinal Berichte* report yaws as very common on Nauru, but during the period of British administration the disease has decreased, only one new case being reported there in 1926. In 1908 Robertson observed that yaws occurred in all of the Gilbert and Ellice Islands, but by 1932-1933 the annual reports referred to the disease as well under control.

In Fiji in the past every child was said to contract yaws. Mortality was very high. However, according to the League of Nations reports for 1937, yaws has been brought under control. In the Wallis and Horn Islands, where it is reported as prevalent, few escape it. In Tonga the infection is lighter than in Fiji and Samoa. In Western Samoa, yaws formerly affected almost all inhabitants, but the treatment program instituted in 1923 has checked it effectively. In American Samoa, 1,040 cases with active lesions were reported in 1929.

More than a thousand cases of yaws were reported on Niue in 1941, but treatment prevented the development of many cases beyond the initial stage. The *Tokelan Medical Survey* of 1927 reports that yaws is not prevalent there and that most cases observed are of the secondary stage.

In the Cook Islands, yaws is said to be more common in the southern than in the northern groups. Treatment programs have been carried out in both groups.

Yaws is reported from Hawaii by both McKinley and the Hawaii Board of Health reports. However, according to Hamlin, Hawaii, like the Marquesas, has escaped infection. Dr. Bernard Witlin, Territorial bacteriologist, reports that Hawaii's few cases are off-shipping, no focus of infection in the group being known.

Gangosa, doubtless a tertiary manifestation of yaws, is reported in New Guinea. Rhinopharyngitis, which also belongs to the yaws-syphilis group, has been known for a long time in the St. Matthias group. Gangosa is by no means uncommon in Papua. It has been observed in the Carolines, Marianas, and Marshalls. Rhinopharyngitis is said to have been described from the Marianas as early as 1600. It is reported on Guam. Gangosa is reported in the Solomons. In Rennel and Bellona Islands in the Solomons, Lambert found yaws but not gangosa. Crichlow, however, reports gangosa from other islands in the Solomons. It is often seen in the New Hebrides, but it is uncommon in the Gilbert Islands. It is reported in Fiji. Rhinopharyngitis is reported in Western Samoa. Gangosa is reported in the Cook Islands.

Goundou, many cases of which seem to fall in the category of yaws sequelae, is reported from New Guinea, New Ireland, the Loyalty Islands, the Solomons, and Samoa.

Juxta-articular nodules, a tertiary manifestation of yaws in the Pacific islands area, were first noted in New Guinea in 1901. Since that time they have been reported also from the Palaus, the Solomons, New Caledonia, the Isle of Pines, the Loyalty Islands, Fiji and Hawaii.

As yaws has long been established in the Pacific Islands where it is believed by Lambert, Hamlin and others to have immunized the native populations against syphilis, it is necessary to examine critically all records of "syphilis" in native islanders. Hamlin has examined numerous reports of syphilis and concludes that many of them refer to yaws. Lambert states that in the examination of large numbers of cases of "so-called syphilis," he was not able to find the scar of a single chancre.

Syphilis has been reported recently as rare or non-existent in the Territory of New Guinea and Papua.

The *Medizinal Berichte* reports many cases of "syphilis," many of them tertiary, from the Palaus, Carolines, and Marshalls; however, where descriptions have been made it appears likely that the

German physicians were dealing with yaws. Robert Koch and Prowazek, who visited the area hospitals, both remarked upon the confusion of yaws with syphilis, while Hamlin, on the basis of his own studies, rejects the records of syphilis in the Marshalls. It is reported that the early English voyagers left syphilis in the Palaus, and natives returning there from other groups are believed to have brought syphilis with them. Soldiers are said to have introduced the disease into the Marianas. Syphilis is reported as practically absent from the Chamorro group on Guam, and it has been stated that no case of syphilis has been acquired by American service men there.

There is said to be no syphilis in the British Solomon Islands, though Hetherington reports a case with a history of exposure on Rennell Island and nowhere else. The British Solomon Islands Protectorate Year Book lists locomotor ataxia as present in the area. In New Caledonia, syphilis is said to be widespread in the penal colonies but rare among the free colonizers. It is reported as rare in the Loyalty Islands.

In the New Hebrides syphilis seems to have increased since the introduction of Indo-Chinese laborers. The *British Annual Report* for 1921-1922 states that it appears to be very common.

The administrator of Nauru reports a number of cases of syphilis treated on that island, but at least some of these were members of crews of visiting ships.

The *Gilbert and Ellice Islands Annual Reports* formerly recorded many cases of "syphilis." More recently it has been emphasized that tertiary yaws has been erroneously reported as "syphilis," and from about 1930 on it is stated each year that syphilis is unknown in these island groups.

There is said to be practically no syphilis among the natives in Fiji but the imported Indian coolie is largely "syphilized." The disease is reported from Tonga.

According to the health reports, syphilis in Western Samoa would seem to be a disease of aliens. The native population of American Samoa is likewise thought to be free, and infected visitors are not allowed to remain on the island.

The Resident Commissioner reported "syphilis" as common on Niue in 1913-1914, a period during which distinctions between tertiary yaws and syphilis were not often drawn. At about the same period many cases were reported from the Tokelau group.

In the Cook Islands, a Hunterian chancre and mucous patches in the mouth co-existing with a typical yaws eruption on the body is reported. The Resident Commissioner reports cases of syphilis, but not in the natives. In Tahiti much of the supposed "syphilis" is believed to be yaws.

The *Pitcairn Medical Report* records a case of syphilis in a woman who may have contracted it in the Gambier group. In 1931, syphilis was reported as present on Easter Island. In Hawaii syphilis is an important civilian and military health problem. There are a few cases of early syphilis reported every month.

#### DISEASES CAUSED BY PROTOZOA

##### *Amoebiasis, Balantidiasis, Giardiasis and Chilomastigiasis*

The distribution of amoebiasis in the Pacific is said to coincide with the distribution of the Asiatic populations, and the disease occurs commonly where large numbers of Indian and Chinese coolies have been imported. It is reported, sometimes sporadically, from New Guinea eastward as far as the Marshalls, Gilbert and Ellice Islands, and Samoa. It is reported also in the Hawaiian Islands.

Figures on the degree of incidence of amoebiasis in the Pacific are not altogether reliable. It has been said that about 50% of the natives and about the same proportion of Europeans in New Guinea suffered from the disease in one form or another, but this figure needs confirmation. Amoebiasis is reported as endemic and one of the principal causes of morbidity throughout all the regions of New Guinea Territory and Papua.

In 1929 it was reported that there were in the British Solomon Islands Protectorate only a few cases of amoebic dysentery, mainly among the Europeans, but it has been recently reported that the disease is widespread and endemic there, and that it assumes epidemic proportions sporadically.

In New Caledonia, amoebiasis is reported as common and endemic. It is said to be increasing yearly there, with the rate of infection reported to be high. The disease is believed to have spread from New Caledonia into the New Hebrides, where it is now endemic throughout the group. In the Gilbert and Ellice Islands it is prevalent and endemic. In Fiji, where the infection is largely endemic, the incidence has dropped. It is reported also in the Hawaiian Islands.

*Balantidium* (Papua, Guam, Japanese Mandated Islands, Western Samoa), *Giardia*, *Trichomonas*, and *Chilomastix* have been reported from the Pacific Islands from time to time.

### *Leishmaniasis*

"Bouton d'Orient," a synonym for cutaneous leishmaniasis, is reported from New Caledonia and the Loyalty Islands. Leishman-Donovan bodies were found in at least one case. The same disease, reported as "Oriental sore," is reported from time to time off shipping by Hawaiian authorities. Three cases of kala-azar (visceral leishmaniasis) in Indians were reported from Fiji in 1924.

### *Malaria and blackwater fever*

Before the present war, malaria and its sole vector, the *Anopheles* mosquito, were known to range through New Guinea Territory, Papua, the British Solomon Islands Protectorate, and the New Hebrides. During the course of hostilities, however, it may well be that this disease and its carrier will extend their range into Pacific islands hitherto free of them.

In New Guinea Territory and Papua, malaria is widespread, especially in the coastal regions. It is reported as common up to an altitude of about two thousand feet, and it occasionally goes higher. *Falciparum*, *malariae* and *vivax* malaria are all present. In the Kavieng District of New Guinea Territory, which includes the northern half of New Ireland and certain adjacent island groups, and which has a total population of about 25,000, nearly 80% of the inhabitants were found to be infected. The 1937-1938 report on New Guinea Territory to the League of Nations described Rabaul as having become definitely malarious, although it had been practically free of the disease during the preceding seven or eight years. In 1942 Rabaul was reported as malaria-free, but it may have become re-infected by now.

The British Solomon Islands Protectorate is heavily infected; few permanent residents, European, Asiatic, or native, escape the malaria. Three forms of the disease occur. *Falciparum* malaria is reported to have become comparatively common, although it was previously rare. About 80% of the natives have palpable malarial spleens. Malaria is reported also in the Santa Cruz Islands. It occurs in all of the inhabited islands of the New Hebrides group with the possible exception of Aneityum and Futuna.

Blackwater fever, a condition invariably associated with malaria, has been reported from Dutch New Guinea, New Guinea Territory, where it is common, and from Papua, the British Solomon Islands Protectorate, New Britain and New Ireland. An imported case was reported in New Caledonia in 1905, but the disease did not become established there.

Blackwater fever was rare in the Solomons before 1915 but it became common thereafter. There are said to be numerous "Blackwater fever houses" there in which repeated cases occur.

Blackwater fever occurs in Europeans in the New Hebrides, where the first case was reported in 1899.

### *Trypanosomiasis*

Typical African trypanosomiasis has been reported in a European in the hospital at Noumea, New Caledonia; the patient had presumably contracted the disease in Africa where the tsetse flies of the genus *Glossina* which transmit the disease occur.

## DISEASES CAUSED BY METAZOA

### *Ancylostomiasis*

The hookworm which occurs most commonly throughout the South Pacific is *Necator*. Cases of *Ancylostoma* infection are not unusual, but they can be linked to relatively recent immigration of Asiatics.

In Dutch New Guinea hookworm was reported in 1935 as widespread. In New Guinea Territory, it infects practically everyone throughout regions under white control, and the prevailing form is *Necator americanus*, with *Ancylostoma* almost completely confined to Asiatics. Hookworm is reported from New Britain, New Ireland, the Admiralty Islands, and Bougainville.

In the Territory of Papua, the hookworms identified have been nearly always *Necator*. Lambert reports that among 2,133 hookworms classified there only 22 *ancylostomes* occurred. Hookworms infect the majority of natives in the wetter part of the country. A few cases of hookworm are reported from Samarai Island, and many from the Trobriand group.

Hookworm is reported from the Palau, Marianas, Carolines, and Marshalls. It is very common on Guam where both *Necator* and *Ancylostoma* are reported.

Bollig's account of a frequently fatal synergism between *ancylostomiasis* and tuberculosis on Truk

may apply also to other islands of the Japanese Mandate.

In the Solomons, *Necator* is endemic. Every island in the Protectorate appears to be infected with hookworm, and the infection rate is estimated at 85% among the natives. It was reported in New Caledonia, probably for the first time, in 1912, and the number of infections there is said to be large and on the increase. It is reported in the Loyalty Islands. In the New Hebrides hookworm is very common and is both endemic and epidemic in type. In 1933 it was almost eradicated from Nauru. Hookworm is more prevalent in the Ellice than in the Gilbert Islands. On Funafuti in the Ellice group, *Necator* is very prevalent; incidence was said to be 85% in 1922-1923. Light infections are reported from Ocean Island.

In 1928, Lambert reported that hookworm in Fiji had been controlled as an economic factor, but that it would never be eradicated until the people acquired a "sanitary conscience."

Rotumah is one of the most heavily infected of the South Pacific Islands. Hookworm is not serious in Tonga however, where pure *Necator* occurs. Lambert reports hookworm as prevalent in Western Samoa, and O'Connor found 85% of the natives there infected. *Necator* is reported from American Samoa.

On Niue, many cases of anemia are caused by hookworm. In 1927 it was reported on Tokelau that no marked clinical evidence could be found of hookworm. The infection is endemic in the Cook Islands, with Tongareva (Penrhyn) and Danger (Puka Puka) in the northern groups, and Rarotonga, Mangaia, and Mauke in the southern group specifically mentioned as infested with *Necator*. No hookworm infection was reported from Pitcairn in 1937. It is reported on Tahiti. Cases are reported regularly from the Territory of Hawaii where both *Ancylostoma* and *Necator* are said to be present. However, most of the hookworm infections are in workers imported from the Philippine Islands rather than in natives.

#### *Ascariasis*

*Ascaris* is reported from New Guinea Territory and Papua. Lambert found it confined to the Delta Division in Papua, with none in the central sections.

In the Japanese Mandated Islands, incidence of *Ascaris* is very high (about 90% according to the Reports to the League). It has been noted in the Palaus, Carolines, Marianas, and Marshalls.

Brain involvement with *Ascaris* is reported in the Marianas. There is said to be a close association between the toxins of intestinal worms, especially *Ascaris lumbricoides*, and mechanical obstruction in the cases of so-called epidemic asthma. However, other factors causing asthma as elsewhere have not been properly worked out.

In the British Solomon Islands Protectorate, *Ascaris* is common, especially in native children. In New Caledonia it was reported in 1926 as very rare, but in 1929, 2,075 examinations showed 12.8% infection. It occurs in the Loyalty Islands. In the New Hebrides *Ascaris* is not found among Melanesians who have not had contact with foreigners.

The administrator of Nauru states that though the Chinese laborers have *Ascaris* the natives have not been infected. Lambert reports that no *Ascaris* infection was found among the Gilbert or Ellice islanders, though "pig ascaris," said to be a different physiological strain, was found in the latter group. In 1935 a number of cases of *Ascaris* were reported from Ocean Island.

In the Fiji Islands incidence is highest among the Indians, and the disease is absent from natives not living in contact with the Asiatic group. Lambert states that no *Ascaris* was found on Rotumah. It has been reported in the Wallis and Horn Islands. In Tonga it is reported as rare on Tongatabu but common in the Vavau group, which has been more closely associated with Samoa. In Western Samoa, ascariasis is said to be very common. It is reported from American Samoa and Niue.

A medical survey of Tokelau found *Ascaris* apparently unknown there. For the Cook Islands an incidence of 21% was reported in 1928, but by 1936 there was on Rarotonga in this group a 55% incidence. *Ascaris* is reported on Tahiti, in the Marquesas, and in the Hawaiian Islands.

#### *Distomiasis (Trematode or fluke infections)*

A case of bilharziasis (schistosomiasis) was reported in the New Hebrides in 1930. McKinley reports it with a question mark from the "French Pacific Group." Hall reports schistosomes as "introduced from time to time" but not established in the Hawaiian Islands.

In New Guinea Territory, cases of *Clonorchis* infection has been reported from New Britain and Bougainville Island. *Clonorchis* has been reported in immigrants in New Caledonia.

The League reports on the Japanese Mandated Islands record a number of cases of hepatic distomes. These may have been *Clonorchis*, or, as the reference to two cases of "*Distoma hepaticum*" suggests, some of them may have been *Fasciola*.

*Fasciola gigantica* has been reported from man in Hawaii. Clonorchiasis has been found in Oriental immigrants in Hawaii. Infection among the natives has been attributed to the eating of fish imported from the Orient.

*Paragonimus* has been reported in New Guinea Territory, and will probably be found elsewhere in the Southwest Pacific. Mumford reports the eggs of a fluke resembling those of *Paragonimus* in the Marquesas. Lambert reports the eggs of a "new trematode" in a fecal specimen from Papua. *Stellantchasmus falcatus*, a heterophyid fluke, is reported established in Hawaii. Infection is obtained by eating raw mullet, a fresh water fish which may harbor the larval trematodes.

Pulmonary distomiasis is reported from the Japanese Mandated Islands and Nauru.

#### *Dracontiasis*

Dracontiasis has been reported from a seaman treated in the Marshall Islands and from Indians in Fiji.

#### *Filariasis and Elephantiasis*

Filariasis of nocturnal periodicity and caused by *Wuchereria bancrofti* occurs in some parts of New Guinea, the Bismarck Archipelago, and the Solomons. It is reported also from the New Hebrides and New Caledonia. In general this form of the disease does not range east of longitude 170°E.

Non-periodic filariasis, also due to *W. bancrofti*, on the other hand, is for the most part limited to the Pacific Islands area east of this line. It prevails in the Gilbert and Ellice Islands, Fiji, Tonga, Wallis, Samoa, Tokelau, the Cook Islands, and the Society Islands. According to Breinl, there may be localities in southern New Guinea where non-periodic filariae also occur.

*Culex quinquefasciatus* is the vector of the microfilariae of nocturnal periodicity, and *Aedes scutellaris pseudoscutellaris* (Syn. *A. variegatus*) is the carrier of the non-periodic microfilariae.

Filariasis occurs fairly commonly in Dutch New Guinea, New Guinea Territory, and the Territory of Papua. In New Guinea Territory filariasis and elephantiasis are reported from the mainland and

from New Britain, Duke of York Islands, New Ireland, the Admiralties, the Ninigo groups, and from Buka and Bougainville Islands. In the Territory of Papua they are reported from the mainland, and from Samarai Island and the Woodlark and Trobriand groups.

Filariasis and elephantiasis are known from the Palau, Caroline, Mariana, and Marshall Islands, but it would seem that in none of these groups are they as common as in Samoa. Non-periodic filariae are said to have been brought to the Marianas by exiled Samoans. Filariasis is reported from Guam, but it is said that elephantiasis does not occur there.

In the British Solomon Islands Protectorate, filariasis, usually nocturnal, is fairly common and widespread, but this is not true of elephantiasis.

Filariasis is prevalent in the Santa Cruz group. In New Caledonia, microfilariae have been reported in the blood of natives, Europeans, and Japanese. Elephantiasis, adenitis, and lymphangitis have been observed there frequently.

Clinical and sub-clinical filariasis are reported from the Loyalty Islands. Elephantiasis is reported on Ouvea, Lifou, and Mare. Cervical adenitis, possibly filarial, has been reported in the Loyalty Islands in children.

Filariasis of nocturnal periodicity is reported in the New Hebrides, where it is said to be common though less so in the southern than in the northern islands. It is caused by *W. bancrofti*, and probably transmitted by *A. scutellaris pseudoscutellaris*. A few cases among the whites are reported. Hydrocele is said to be common.

On Nauru, filariasis is endemic and occasionally mildly epidemic. *Wuchereria bancrofti* is the parasite, and *Culex quinquefasciatus* has been shown to carry it. The percentage of infection has risen considerably since 1932.

The Gilbert Islands are described as comparatively immune. Cases of filariasis are reported on Nukunau, however, and a survey in 1938 reports filariasis on Tarawa, Maiana, Apaiang, and Butaritari, in order of diminishing intensity, but not on Makin Island. According to Lambert, it is non-periodic in the Gilberts, and a 1924-1925 report stated that "until recent times there was no history of filarial fever and no elephantiasis" there.

Filariasis is very common in the Ellice Islands, especially on Nui, Vaitupu, and Niutao. Embryos have been reported in the blood of every inhabitant



of Funafuti in the Ellice Islands. Many cases of elephantiasis are reported from that group. Filariasis is among the diseases that were known to the natives when Fiji was discovered by the Europeans. It is found chiefly among the natives and in certain districts it is quite common. Elephantiasis of the arms is the most common manifestation. Chyluria is reported there.

Filiariasis and some elephantiasis are present in the Wallis and Horn Islands. In the Wallis Islands half of the population is infected. Filariasis is reported as present throughout Tonga also.

In Samoa, filariasis is non-periodic. It is reported as very common in Western Samoa. Elephantiasis is common, and chyluria is recorded. Filariasis is less common in American Samoa than in Western Samoa, but it is present there along with elephantiasis.

On Niue filariasis is reported as fairly common, the second most important health problem.

Reports from Tokelau are conflicting. The Tokelau Medical Survey of 1927, for example, states that owing to the absence of mosquitoes in the village inlets, filariasis is not common there. In *Maladies Exotiques*, 1928, however, filariasis is said to be common in certain parts of Tokelau, and the Samoan Administrator has reported a few Tokelau natives as suffering from elephantiasis.

Lambert found both filariasis and elephantiasis spread through the Cook Islands, cases of filariasis being reported on Rarotonga, Mangaia, Mauke, Aiutaki, and Atiu Islands.

Filariasis is common in the Society Islands, where elephantiasis also occurs. In 1833 the total absence of elephantiasis from the Marquesas was commented upon, but by 1843 it was reported as present on Nukuhiva.

Though *Filaria* is often found in Hawaii, usually in the blood of Samoan immigrants, clinical elephantiasis is not common there. Both elephantiasis and chyluria are recorded in Hawaii; the first by the Board of Health and the second by McKinley.

#### *Myiasis and larva migrans*

Myiasis in man are reported from New Britain, New Ireland, and the Solomons. Larva migrans is reported in New Guinea.

#### *Oxyuriasis*

*Enterobius* is reported from New Guinea Territory, the Territory of Papua, the British Solomon

Islands Protectorate, the New Hebrides, New Caledonia, Nauru, the Gilbert and Ellice Islands, Fiji, the Cook Islands, and Hawaii.

*Enterobius* is reported from the Japanese Mandated Islands and Guam.

*Enterobius* is present in the Hawaiian Islands, but the incidence there is unknown, since routine fecal examinations detect only a small proportion of cases. It occurs in the Marquesas.

#### *Strongyloidiasis*

*Strongyloides stercoralis* is reported from New Guinea Territory, the Territory of Papua, the British Solomon Islands Protectorate, the New Hebrides, New Caledonia, Fiji, American Samoa, and Hawaii. It occurs frequently on Guam.

#### *Taeniasis*

Unspecified tapeworms have been reported from northern New Guinea, New Britain, New Caledonia, and Tahiti. The reports to the League of Nations on the Japanese Mandated Islands mention them, but they have not been found on Guam.

*Diphyllobothrium latum* has been reported from Papua.

*Hymenolepis nana*, the dwarf tapeworm is reported from New Guinea, Papua, Nauru, the New Hebrides, Fiji, and the Hawaiian Islands. According to Hall, this is the common tapeworm of Hawaii.

*Taenia saginata*, the beef tapeworm of man, is reported from New Britain, the Carolines, the Marshalls, Fiji, and Hawaii. It is not present in New Guinea and Papua.

*Taenia solium*, the pork tapeworm of man, is reported from Western Samoa, Nauru, and the Hawaiian Islands. It is not present in New Guinea and Papua.

#### *Trichinosis*

In 1941 the incidence of *Trichinella spiralis* in the Hawaiian Islands was reported as constituting an important public health problem there. Alicata in 1938 reported a high incidence (15 per cent) of infection among wild hogs on Hawaii, and since a large number of these animals are used for human consumption they are considered important in connection with human trichinosis there.

#### *Trichostrongylus infection*

*Trichostrongylus* infection is reported from Fiji and Hawaii.



*Trichuriasis*

*Trichuris* is reported also from New Guinea Territory, the Territory of Papua. A 90% infection-rate is reported on Guam, the British Solomon Islands Protectorate, the New Hebrides, New Caledonia, Fiji, Gilbert and Ellice Islands, Rotumah, Samoa, Tonga, the Cook Islands, and Tahiti. According to clinicians, there is a relatively high incidence of *Trichuris* in the Hawaiian Islands.

*Scabies and other acariases*

In New Guinea Territory, both scabies and *casca*s, which appears to be a complex of *Sarcoptes* infection with bacterial invasion, are well known. Scabies is reported from the Territory in Northern New Guinea, New Britain, New Ireland, and the St. Matthias Islands. It is reported also from the Territory of Papua and from Samarai Island.

A red mite infestation often followed by ulceration at infection sites is reported as common in New Guinea. *Microthrombidium wichmanni* Oudemans and *Schöngastia vandersandei* Oudemans are reported as affecting man there. In tropical Australia and in Melanesia there are (in addition to *Sarcoptes scabiei*) various velvet mites which cause severe itching to the human skin. In Dutch New Guinea and New Guinea Territory Cilento reports various mites which establish themselves in lesions in the skin but appear to be something other than *Sarcoptes*. A case of mites, possibly *Rhizoglyphus* sp., excised from skin tissue is reported from New Guinea Territory.

Scabies is reported from the Palau and from Yap and Ponape Islands in the Carolines. *Sarcoptes* is reported in the Carolines and Marshalls. Infestations by a harvest mite, probably *Trombicula* sp., in the Palau and Caroline Islands, and by a *Tyroglyphus* on Truk, in the Carolines are reported.

Scabies is reported as common in the British Solomon Islands Protectorate. In the Loyalty Islands, it is reported from Lifou, Mare, and Ouvea. It is frequent in the New Hebrides.

Cases of scabies in natives and Chinese are reported from Nauru. The infection is very common in the Gilbert and Ellice Islands, especially in children. In Fiji it is common among the natives. It is widespread on Wallis and Horn Islands. In Western Samoa and Niue it is reported as common. In the Cook Islands, the Resident Commissioner states that it was probably introduced into Palmerston Atoll from French vessels stranded there in 1913, that it has been a "plague" on Atiu, and that it is common on Rarotonga. In the Marquesas the common infection *puera* bears a marked resemblance to it. Scabies is reported also from Pitcairn.

Scabies is reported from the Hawaiian Islands by the Hawaii Board of Health. In Hawaii skin rashes are reported to be caused by *Liponyssus bursa* and *Pediculoides ventricosus*. Rat mites of the genus *Laelaps* are considered as potential pests, but have not as yet been reported as infesting man. No Trombidiids have been found there.





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*Editor, CHAS. F. CRAIG*  
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ROBERT BRIGGS WATSON, Tennessee Valley  
Authority, Wilson Dam, Alabama.

JOHN F. KESSEL, Univ. Southern Calif.,  
School of Medicine, Los Angeles, Calif.

OLIVER R. MCCOY, School of Medicine &  
Dentistry, University of Rochester,  
Rochester, N. Y.

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# RENEWED CLINICAL ACTIVITY IN NATURALLY INDUCED VIVAX MALARIA\*

MARK F. BOYD AND S. F. KITCHEN

*Station for Malaria Research, Tallahassee, Florida*

In a previous paper (1) we presented observations on the renewal of clinical activity in infections with the McCoy strain of *Plasmodium vivax*, experienced by 149 patients who had been naturally inoculated with parasites of this strain up to the end of 1935. The material enlargement of our series of inoculations now makes desirable a further analysis of the circumstances under which renewed or 'secondary clinical activity has been observed.

## MATERIAL

In slightly more than 10 years time (June 12, 1931, to August 1, 1941) there were on the malaria therapy service of the Florida State Hospital, 388 patients, all white, successfully inoculated with vivax malaria by the application of infected mosquitoes. Of these patients, 375 experienced attacks of clinical malaria in varying degree and 13 exhibited only a transient parasitemia. The experience of the former group is the subject of our analysis.

## REMISSIONS AND THERAPEUTIC INTERFERENCE

In Table 1 the duration of the primary clinical activity (until either its spontaneous termination or its induced termination by therapeutic interference) is compared with the frequency thereafter of secondary clinical activity, assuming that remissions of 5 or more days may have marked the end of the primary attack. It will be noted that such secondary clinical activity was experienced by 105 (40.2 per cent) of 261 patients with spontaneous remissions and by 59 (51.8 per cent) of 114 patients in whom a remission resulted from some degree of therapeutic interference. Secondary clinical activity was not noted in untreated patients whose primary attack exceeded 55 days in duration, or following attacks which had proceeded for 45 days before interruption. The latter attacks, but for the interruption, may be regarded as of potentially longer duration, although

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the means of the actual clinical activity experienced do not differ significantly.

In Table 2 is summarized the extent of the therapeutic interference with the primary attack, and with successive secondary attacks as well, up to and including the third. Full therapy signifies the administration of either a total of 14 grams or more of quinine or 1.5 grams of atabrine, in divided doses in either case. In general the interrupting or minor doses have been of quinine, in amount usually not exceeding a total of 2 grams. It will be noted that when termination of the primary attack was spontaneous, secondary clinical activity occurred only among those patients (46.5 per cent) who did not receive subsequent therapy. Interruption of the primary attack or an induced termination, without subsequent full therapy, resulted in the reactivation of a larger proportion (73.0 per cent). Many experienced a further series of secondary attacks, although at a decreasing rate for the corresponding class. When therapeutic interference was followed by full therapy, secondary activity was noted only in from 21 to 25 per cent of the patients.

The frequency of secondary attacks according to (a) all patients, (b) the proportion which any group of such events bears to the whole, and (c) the proportion of patients who, having experienced one, experience another, is derived from Table 1 and expressed in percentages as follows:

*Percentages of Secondary Clinical Activity According to Termination of Primary Attack*

NUMBER OF SECONDARY ATTACKS	SPONTANEOUS			INDUCED		
	Among all patients	Of all second- ary at- tacks	Of pre- ceding group	Among all patients	Of all second- ary at- tacks	Of pre- ceding group
1	40.2	100.0	40.2	51.8	100.0	51.8
2	14.2	35.2	35.2	25.4	49.2	49.2
3	3.4	8.6	24.3	9.6	18.6	37.9
4	1.1	2.6	33.3	2.6	5.1	27.3
5	0.4	1.0	33.3	1.8	3.4	66.6
6				0.9	1.7	50.0

From this it appears that the interrupted infections present a larger proportion of secondary

attacks. Reactivating patients in this category had a mean of 1.78 such secondary attacks, as secondary attacks probably represent a class not

TABLE 1

*Duration of Primary Attack of Malaria and Frequency of Secondary Attacks in 164 Patients Experiencing Secondary Attacks*

PRIMARY ATTACK DURATION OR INTERVAL TO FIRST REMISSION	PATIENTS		SECONDARY ATTACKS												TOTAL	
	All	Primary alone	1		2		3		4		5		6		Attacks	Patients
			All	Only	All	Only	All	Only	All	Only	All	Only	All	Only		
<i>days</i>																
Termination:																
Spontaneous																
0-7	22	17	5	3	2	2									7	5
7-13	74	56*	18	12	6	3	3	2	1		1	1			29	18
14-20	48	13*	35	19	16	12**	4	2	2	2					57	35
21-27	30	14	16	12	4	3	1	1							21	16
28-34	29	16	13	8	5	5									18	13
35-41	22	13	9	8	1	1									10	9
42-48	20	12	8	5	3	2	1	1							12	8
49-55	9	8	1	1											1	1
56-62	5	5														
63-69	2	2														
Total.....	261	156	105	68	37	28	9	6	3	2	1	1	0	0	155	105
Induced																
0-7	13	10*	3	2	1	1									4	3
7-13	15	6*	9	4	5	4	1		1		1		1	1	18	9
14-20	31	15***	16	9	7	5	2	2							25	16
21-27	16	7*	9	4*	5	2	3	2	1		1	1			19	9
28-34	21	9	12	6	6	2	4	3	1	1					23	12
35-41	12	5	7	2	5	4	1	1							13	7
42-48	6	3	3	3											3	3
Total.....	114	55	59	30	29	18	11	8	3	1	2	1	1	1	105	59
All patients.....	375	211		98		46		14		3		2		1		164
All secondary.....			164		66		20		6		3		1		260	
Each star represents one death within 6 mos. any cause .....		8		1		2										11

*Mean duration of primary attack*

TERMINATION	WITH PRIMARY ATTACK ONLY	WITH SECONDARY ATTACK	TOTAL
Spontaneous.....	23.2 ± 1.1	22.7 ± 1.4	23.0 ± 0.9
Induced.....	20.8 ± 1.9	23.6 ± 1.8	22.2 ± 1.3
Total.....	22.6 ± 1.0	23.0 ± 1.1	22.8 ± 0.7

compared with a mean of 1.48 in those whose primary attacks ceased spontaneously. infrequently noted among treated autochthonous infections. The patient who had six is a young

white male, whose primary attack was interrupted by 1 gram of quinine on the 18th day (after inoculation). Repeated resumption of clinical activity lead to further interruptions, as follows:

- (1) On the 30th day by 0.6 gram quinine
- (2) On the 56th day by 1.5 gram atabrine (full course)
- (3) On the 145th day by 1.5 gram atabrine (full course)
- (4) On the 204th day by 14 grams quinine (full course)
- (5) On the 305th day by 14 grams quinine (full course), and finally

particular period of the illness. Clinical activity was terminated in 77.8 per cent of the patients receiving full therapy; while in 29.0 per cent of those receiving therapeutic interference, even the small doses effected a termination rather than a remission. It does not appear that the period of the primary attack at which therapy of any kind was initiated influenced the likelihood of bringing about a termination.

The duration of the first remission following different methods of therapeutic interference with the primary attack of 114 patients is shown in Table 4. While in 18 instances small doses of

TABLE 2

*Relation of Therapy to Termination of Primary Attack of Malaria and to Secondary Activity*

TERMINATION	SUBSEQUENT THERAPY	PRIMARY ATTACK			SECONDARY ATTACKS								
					1st			2nd			3rd		
		All	Further		All	Further		All	Further		All	Further	
			No.	P.c.		No.	P.c.		No.	P.c.		No.	P.c.
Spontaneous	None	226	105	46.5	82	37	45.1	31	9	29.0	7	3	42.9
	Quinine												
	Minor	2	0		3	0		1	0				
	Full therapy	30	0		18	0		5	0				
	Atabrine	3	0		1	0					2	0	
	Other				1	0							
Total.....		261	105	40.2	105	37	35.2	37	9	24.3	9		
Induced	None	63	46	73.0	32	17	53.1	22	9	40.8	8	2	25.0
	Quinine												
	Minor	2	1		8	6	75.0	1	1		2		
	Full therapy	37	8	21.6	15	4	26.7	5	0				
	Atabrine	8	2	25.0	3	1		1	1		1	1	
	Other	4	2	50.0	1	1							
Total .....		114	59	51.6	59	29	49.3	29	11	37.8	11	3	27.3

- (6) On the 415th day by 1.5 gram atabrine (full course) which terminated the infection.

In Table 3 the incidence of the earliest secondary activity in 114 patients following (a) full therapy and (b) interrupting doses of various drugs is compared with the time of interference with the primary attack according to (a) days elapsing since inoculation and (b) days elapsing since clinical onset. Interference was practiced at any stage of the primary attack, but the close approximation of the mean days elapsing to either full therapeutic or interfering doses, indicates there was not any tendency to apply one rather than another at any

quinine definitely terminated clinical activity, reactivation in most instances (39) occurred after remissions not exceeding 4 weeks in duration. On the other hand the 10 remissions produced by full courses of therapy ranged from 6 to 36 weeks in duration. When the effect of treatment is the production of a remission only, the duration tends to vary directly with the quantity of parasitocidal drug administered. The longest remission observed was of 282 days.

#### CHARACTERISTICS OF REMISSIONS

In Tables 5A and 5B there is presented a correlation of the duration of the first, second, and



TABLE 3

*Effect of Therapeutic Interference at Different Periods with Primary Attack of Malaria in 114 Patients*

INTERVAL IN	RESULT	TO INTERFERENCE										TOTAL	
		0-7	7-13	14-20	21-27	28-34	35-41	42-48	49-55	56-62	63-69	No.	P.c.
A. Days from inoculation: Full therapy begun	Terminating Not terminating			6 2	**9 1	**8 2	2 2	6 1	1 2	2	1	35 10	77.8
Total .....				8	10	10	4	7	3	2	1	45	
Interrupting dose	Terminating Not terminating		1	5 5	4 6	1 12	4 10	5 7		6 3		20 49	29.0
Total .....			1	10	10	13	14	12	6	3		69	
B. Days from clinical onset: Full therapy begun	Terminating Not terminating	4	**6 2	**12 2	3 1	5 3	3 2	2				35 10	77.8
Total .....		4	8	14	4	8	5	2				45	
Interrupting dose.....	Terminating Not terminating	3 2	3 6	4 15	3 9	6 6	1 7		1 3			20 49	29.0
Total .....		5	9	19	12	12	8	3	1			69	

From inoc.: Mean days to full therapy .....  $33.4 \pm 1.9$   
 Mean days to interrupting therapy .....  $34.5 \pm 1.5$  12.49 S.D.†

From onset: Mean days to full therapy .....  $20.3 \pm 1.7$   
 Mean days to interrupting therapy .....  $24.0 \pm 1.4$  11.55 S.D.†

Each star represents one death within three months following interruption.

† Standard deviations used for computing standard errors.

TABLE 4

*Duration of First Remission Following Therapeutic Interference with Primary Attack in 114 Patients*

DRUG	AMOUNT	ATTACK TERMINATED	DURATION REMISSION IN DAYS†																TOTAL			
			0-7	7-13	14-20	21-27	42-48	63-69	98-104	105-111	168-174	182-188	203-209	210-216	224-230	231-237	238-244	245-251	280-286	Patients	With secondary attacks	P.c.
Quin ine	Less than 14 gms.	18*	8	16	12	3	1		1	1	1		2			1			1	65	47	72.3
	14 gms. + 1.5 gms.	29*					1		1			1	1		1		1	2		37	8	21.6
Atabrine		6†						1						1						8	2	25.0
Others		2		2																4	2	50.0
Total .....																				114	59	51.8

\* 1 death within 3 months following interruption.

† 2 deaths within 3 months following interruption.

‡ Columns containing missing spans of days omitted because of absence of observations falling within those limits.

third periods of secondary clinical activity, with the duration of the remissions which preceded them, further distinguishing between spontaneous (A) and

secondary clinical activity rarely exceeded 3 weeks (mean duration 6.9 days). When the remissions were induced (usually the first), 15 pa-

TABLE 5A

*Comparison of Duration of Remissions and Terminating Clinical Activity in 105 Patients  
A. Prior Remissions Spontaneous*

DURATION PRIOR REMISSION	DURATION SECONDARY ATTACK IN DAYS															TOTAL
	0-7			7-13			14-20			21-27			28-34			
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	
days 0-7	24	6	2	7	1	1	3			2			1			37 7 3
7-13	22	23	4	13	1		8	1		2						45 25 4
14-20	8	1		3												11 1
21-27	7	2	1	1												8 2 1
28-34		1	1													1 1
35-41	2															2
42-48		1														1
49-55				1												1
56-62	1															1
Total: 1st .....	64			25			11			4			1			105
2nd .....		34			2			1								37
3rd.....			8			1										9

Remissions: Mean duration in days 1st.....  $11.1 \pm 0.9$   
 2nd.....  $11.0 \pm 1.5$  9.23 S.D.\*  
 3rd.....  $11.8 \pm 3.1$

Secondary attacks: Mean duration in days 1st.....  $6.9 \pm 0.6$   
 2nd.....  $3.6 \pm 0.9$  5.64 S.D.\*  
 3rd.....  $2.6 \pm 1.9$

\* Standard deviations used for computing standard errors.

induced (B) remissions. It will be noted that most of the spontaneous remissions did not exceed 3 weeks in duration (mean of the first 11.1 days), and none exceeded 62 days. The earliest

tients exceeded the maximum noted for the spontaneous remissions, and the mean duration for the group was consequently increased to 60.4 days. The mean duration of the subsequent clinical

activity was 7.7. days, slightly more than in the prior group. The mean duration of the second and third spontaneous remissions did not differ materially from that noted for the first, although

They were slightly longer in the group with induced remissions (5.9 and 6.5 days respectively).

Since the longest remission arising spontaneously (Table 5A) lasted for 61 days, remissions following

TABLE 5B  
*Comparison of Duration of Remissions and Terminating Clinical Activity in 59 Patients*  
*B. Prior (1st) Remission Induced*

DURATION PRIOR REMISSION	DURATION SECONDARY ATTACK IN DAYS															TOTAL		
	0-7			7-13			14-20			21-27			28-34					
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
days																		
0-7	7	3	1	2		1		1				1				9	4	3
7-13	5	11	3	8	5	1	4		1							17	16	5
14-20	6	1	1	4	1					1			1			12	2	1
21-27	4															4		
42-48	1	1					1									2	1	
56-62		1															1	
63-69	1	1														1	1	
77-83			1															1
98-104	1															1		
105-111	1															1		
154-160											1						1	
168-174				1												1		
182-188									1			1				1		
189-195									1								1	
203-209	2			1												3		
210-216	1															1		
224-230	1	1														1	1	
231-237	1															1		
238-244				1												1		
245-251	2															2		
252-258		1															1	
280-286	1		1													1		1
Total: 1st.....	34			17			5			2			1			59		
2nd.....		20			6			2			1						29	
3rd.....			7			2			1			1						11

Remissions: Mean duration in days 1st..... 60.4  $\pm$  10.6  
2nd..... 41.8  $\pm$  15.1 81.33 S.D.\*  
3rd..... 40.2  $\pm$  16.4

Secondary attacks: Mean duration in days 1st..... 7.7  $\pm$  1.0  
2nd..... 5.9  $\pm$  1.5 8.01 S.D.\*  
3rd..... 6.5  $\pm$  2.4

\* Standard deviations used in computing standard errors.

probably significantly shorter than in the interrupted group. The second and third periods of clinical activity by which the remissions terminated were shorter (3.6 and 2.6 days respectively) and were frequently limited to a single paroxysm.

therapeutic interference (Table 5B) may be roughly divided into those of 61 days or less, and those of over 61 days duration. There are 77 of these events in the former category and 22 in the latter, the actual limits of which range from

67 to 282 days. The mean duration of the categories thus subdivided is shown below.

*Mean Duration of Remission in Days*

	INITIATION SPONTANEOUS (NONE OVER 61 DAYS) MEAN DAYS	INITIATION INDUCED		
		All	61 days or less	More than 61 days
1st	11.1 $\pm$ 1.1	60.4	13.9 $\pm$ 1.7	196.6
2nd	11.0 $\pm$ 1.9	41.8	12.4 $\pm$ 2.1	180.6
3rd	11.8 $\pm$ 3.9	40.2	8.6 $\pm$ 3.5	

(Standard deviation of 11.59 days used for computing standard errors.)

The close approximation of the mean duration of the remissions of 61 or less days in length in patients with interrupted attacks, to the mean duration of those occurring spontaneously, may justify an assumption of their similarity, and serve to emphasize the dissimilarity of those which are of longer duration.

As is well known, most vivax-infected patients experience quotidian intermittent paroxysms, which are due to the successive and alternating maturation of two broods of parasites. It is usually feasible to distinguish these as odd or even, depending on whether the paroxysms they produce recur on odd-numbered or even-numbered days elapsing since inoculation. If the density of the parasites of one brood becomes reduced below the pyrogenic level currently prevailing in the patient, the corresponding paroxysm will be suppressed and the attack will change in character from a quotidian intermittent to a tertian intermittent. If both broods are reduced, a remission results. Thus one may assume that any remission which lasts, for example, for 5, 7, 9, \*, 21, \*, or 61, or any subsequent odd number of days is terminated by the reactivation of the same brood of parasites whose original suppression produced it. On the other hand, if the remission lasts for 6, 8, 10, \*, 62, or any subsequent even number of days, it is terminated by the reactivation of the alternate brood. Of 260 remissions considered, 153 terminated on odd days, 107 on even. It is therefore likely that the clinical activity which terminates a remission is more often due to the reactivation of the brood through the suppression of which it arose. On the other hand, of the 24 remissions exceeding 61 days in length, 9 terminated on odd days and 15 on even days, indicating that the termination of these long remissions more commonly marks the activation of the alternating brood.

#### CHARACTERISTICS OF SECONDARY CLINICAL ACTIVITY

The character of the clinical activity during the primary attack and the various secondary attacks is shown in Table 6.

While these data may suggest that the primary attacks which came to a spontaneous termination were essentially milder, as evidenced by the smaller proportion of patients who experienced quotidian paroxysms during their illness, and the larger proportion whose attacks were tertian throughout, or changed from quotidian to tertian, as compared with the interrupted attacks, the differences are not significant. On the other hand, when activity was resumed after the several remissions, the secondary attacks following spontaneous termination of the primary, significantly exhibited a larger proportion of quotidian and a smaller proportion of tertian paroxysms than are noted upon reactivation after the interrupted primary attacks. Evidently the effect of the therapeutic interference in the latter group was not equally deleterious to both broods of parasites.

#### CHRONOLOGICAL FREQUENCY OF SECONDARY ACTIVITY

In Tables 7A and 7B are presented the intervals elapsing from (a) the day of inoculation, (b) the day of onset, and (c) the terminal day of the primary attack (each counted as zero day), to the onsets of the consecutive classes of secondary attacks, further distinguishing whether the earliest remission occurred spontaneously (a) or was therapeutically induced (b). The data have been condensed as follows:

TERMINATION OF PRIMARY ATTACK	SEQUENCE OF SECOND- ARY ATTACK	DAY FROM INOCULATION TO ONSET OF SECONDARY CLINICAL ACTIVITY			
		21-132	133-174	175-202	203-419
Spontaneous	1st	104			1
	2nd	37			
	3rd	9			
	4th	3			
	5th	1			
Induced	1st	45	2		12
	2nd	20	2		7
	3rd	7	1		3
	4th	1			2
	5th	1			1
	6th				1

All instances of renewed activity after spontaneous remissions (with one exception in a pa-

tient with a protracted incubation period) were initiated either within 132 days following inoculation, 118 days following the onset, or 90 days following termination of the primary attack. This, we believe, indicates that all clinical activity observed within these limits is actually a manifestation of the primary parasitemia, and hence should be regarded as a continuation of the primary attack (2). It will also be noted that 70.5 per cent of the instances (74 of 105) of secondary activity following therapeutic interference, also arose within an identical period, which we believe suggests that this fraction has a significance

whose primary attack ceased spontaneously (see text figure). Consequently it would seem that the secondary attacks directly related to the primary parasitemia may arise as late as the 174th day following inoculation, the 160th day following the onset, or the 125th day following termination of the primary attack. The approximate initial limit of the second frequency distribution is about the 204th day following inoculation.

The limitation, in this series, of all instances of clinical reactivation after protracted remissions to patients whose primary attacks were therapeutically interrupted, is significant.

TABLE 6  
*Characteristics of Clinical Activity*

TERMINATION PRIMARY	CHARACTER OF PAROXYSMS	PRIMARY ACTIVITY		SECONDARY ACTIVITY							
		Cases	P.c.	1st	2nd	3rd	4th	5th	6th	Total	
										No.	P.c.
Spontaneous	Quotidian	66	62.9	50	14	3				67	43.2
	Quotidian to tertian	24	22.9	7	1					8	5.2
	Tertian	15	14.3	28	9	1	1			39	25.2
	Tertian to quotidian			1						1	0.1
	One only			19	13	5	2	1		40	25.8
Total.....		105		105	37	9	3	1		155	
Induced	Quotidian	46	78.0	15	5	4				24	22.9
	Quotidian to tertian	8	13.6	5						5	4.8
	Tertian	5	8.5	35	18	5	2	2	1	63	60.0
	Tertian to quotidian			2	1					3	2.9
	One only			2	5	2	1			10	9.5
Total.....		59		59	29	11	3	2	1	105	

similar to the former. The remaining 31 secondary attacks following induced remission began subsequent to the 132nd day, but of these 26 began subsequent either to the 203rd day following inoculation, the 189th day following the onset, or the 168th day following termination of the primary attack. None was noted to arise between the 175 and 202nd days after inoculation. In two instances clinical reactivation has been observed after the lapse of more than one year from the date of inoculation.

It is thus apparent that the onsets of secondary attacks in the interrupted group show two distinct frequency distributions, the first of which closely coincides with, although it slightly exceeds, the only frequency distribution presented by the group

Attention should be called to two patients of the series in whom the prepatent and incubation periods were 304, 304 and 283, 302 days respectively. The coincidence of these intervals to the last frequency mentioned above, suggests that there may be an underlying relationship to the protracted remissions just discussed.

#### THE MCCOY STRAIN

The infections considered were produced by the McCoy strain of *Plasmodium vivax* and 9 other strains of the same parasite, as shown in Table 8. The frequency of secondary activity in McCoy infections subsequent to primary attacks is not significantly different from that in all the other strains observed, nor, when interrupted thera-

peutically, does further clinical activity appear to be more likely with the McCoy strain. Renewed activity followed in 44.1 per cent of all McCoy strain inoculations, and occurred in 40.9 per cent when other strains were used.

The McCoy strain produced most of these infections, and was the only strain routinely propagated throughout this period. During approximately ten years, as shown in Table 9, this strain was passed through 60 consecutive and unbroken human-anopheline transfers, averaging

is not apparent that their incidence was materially affected by the season in which the inoculation was effected.

#### SIMULTANEOUSLY ACQUIRED DOUBLE INFECTIONS

The analyses just discussed related to inoculations effected with *P. vivax* alone. A limited series of cases already reported (3, 4) suggests that in persons simultaneously infected with *P. vivax* and *P. falciparum* the proportion experiencing secondary vivax attacks subsequent to the 175th

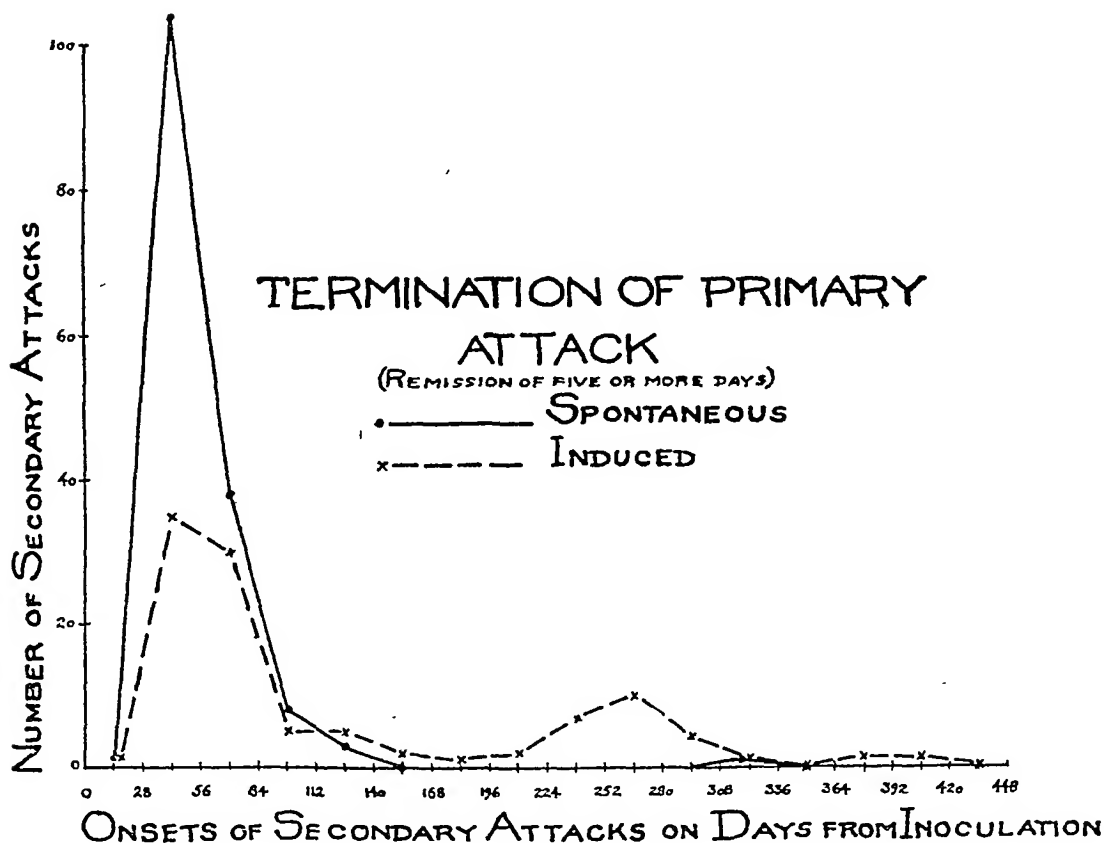


FIG. 1

6 passages per year. We did not observe any change during this time in either the characters of the parasites or the infections which they produced. It does not appear that periods of renewed clinical activity following either spontaneous or induced termination of the primary attack become either more or less frequent as the chain of passage extends.

The season of inoculation with the McCoy strain is considered in relation to the frequency of secondary periods of clinical activity in Table 10. It

day following inoculation may be substantially higher than when *P. vivax* is present alone.

It may be recalled that following these double inoculations, the prepatent periods of the falciparum and vivax infections were of normal limits, but the appearance of falciparum always preceded that of vivax. Except in two instances, the density of the falciparum parasites early outstripped that of the vivax, so that the former parasites immediately dominated the clinical picture, requiring one or more small doses of quinine to restrain their

exuberance in four patients. In one other patient the clinical activity ascribable to the falciparum parasite was mild, and on the point of spontaneous cessation, when 8 days after the initial falciparum rise, the vivax infection began to flourish, and had

parum, but soon succumbed differentially to the action of the small doses of quinine required to restrain the latter, and quickly receded without manifesting any effect on the clinical picture. Thus in every instance with therapeutic inter-

TABLE 7A  
*Interval from Various Events to Initiation of Secondary Activity  
After Spontaneous Remissions*

DAYS	DAYS TO ONSET SECONDARY ATTACK					TOTAL
	0-27	28-55	56-83	84-111	112-139	
From inoculation						
1st.....	1	84	16	2	1	104*
2nd.....		20	14	3		37
3rd.....			7	1	1	9
4th.....			1	2		3
5th.....					1	1
Total.....	1	104	38	8	3	154*
From onset primary attack						
1st.....	39	57	8		1	105
2nd.....	2	25	10			37
3rd.....		4	4	1		9
4th.....			2	1		3
5th.....				1		1
Total.....	41	86	24	3	1	155
From termination primary attack						
1st.....	101	3	1			105
2nd.....	31	5	1			37
3rd.....	1	7		1		9
4th.....		1	2			3
5th.....				1		1
Total.....	133	16	4	2		155

*Mean days to secondary clinical activity*

	1st	2nd	3rd	
From inoculation.....	46.0 ± 1.8	58.6 ± 3.0	75.7 ± 6.2	18.50 S.D.†
From onset primary attack.....	34.0 ± 1.8	46.8 ± 3.0	61.7 ± 6.0	18.13 S.D.†
From termination primary attack.....	11.1 ± 1.6	25.6 ± 2.6	35.9 ± 5.3	16.01 S.D.†

\* Add one patient with incubation of 302 days.

† Standard deviations used for computing standard errors.

just initiated clinical activity when therapeutic termination of the malaria was required because of an intercurrent amebiasis. The vivax parasites in the remaining two patients initially exhibited a capacity to increase at the same rate as the falciparum,

but soon succumbed differentially to the action of the small doses of quinine required to restrain the latter, and quickly receded without manifesting any effect on the clinical picture. Thus in every instance with therapeutic inter-

vivax, which occurred before these patients received full courses of therapy. These vivax attacks subsided spontaneously, and shortly there-

was given to terminate the falciparum infection. In three of these, secondary clinical activity was experienced beginning the 247th, 247th, and 295th

TABLE 7B  
*Interval from Various Events to Initiation of Secondary Activity*  
After at Least One Induced Remission

DAYS	DAYS TO ONSET SECONDARY ATTACK															TOTAL
	0-27	28-55	56-83	84-111	112-139	140-167	168-195	196-223	224-251	252-279	280-307	308-335	336-363	364-391	392-419	
From inoculation																
1st.....	1	29	13	1	2		1	1	4	6		1				59
2nd.....		5	12	1	2	2			3	3	1					29
3rd.....		1	5	1	1					1	1			1		11
4th.....				1				1			1					3
5th.....				1							1					2
6th.....															1	1
Total.....	1	35	30	5	5	2	1	2	7	10	4	1		1	1	105
From onset primary attack																
1st.....	8	32	4	1	1	1	1	2	5	3	1					59
2nd.....		10	7	1	3	1		2	4	1						29
3rd.....		1	6		1					1	1			1		11
4th.....			1				1				1					3
5th.....				1							1					2
6th.....															1	1
Total.....	8	43	18	3	5	2	2	4	9	5	4			1	1	105
From termination primary attack																
1st.....	42	2	1	2			2	4	5		1					59
2nd.....	9	8	2	1	2			4	3							29
3rd.....	1	6			1					1	1		1			11
4th.....		1					1			1						3
5th.....			1								1					2
6th.....															1	1
Total.....	52	17	4	3	3		3	8	8	2	3		1		1	105

*Mean days to secondary clinical activity*

	1ST	2ND	3RD	S.D.*
From inoculation.....	94.7 ± 12.4	121.0 ± 17.6	152.3 ± 28.7	95.02 S.D.*
From onset primary attack.....	79.3 ± 12.4	107.9 ± 17.6	135.0 ± 28.6	94.96
From termination primary attack.....	76.1 ± 12.7	87.0 ± 18.2	110.4 ± 29.5	97.90

\* Standard deviation used for computing standard errors.

after the full courses of therapy mentioned were given. No further clinical activity occurred. In the remaining four patients, no indication of a reactivation of vivax was noted before full therapy

days after inoculation, with further activity occurring in two beginning with the 268th and 274th days. In our opinion this experience is attributable to the full therapy.



TABLE 8  
*Frequency Secondary Attacks with Different Strains*

TERMINATION	STRAIN	NO SECONDARY ATTACKS	SECONDARY ATTACKS						TOTAL
			1	2	3	4	5	6	
Spontaneous	McCoy 8 other	139	57	24	6	2	1		229
		17	11	4					32
Total.....		156	68	28	6	2	1		261
Induced	McCoy 9 other	46	27	18	8	1	1	1	102
		9	3						12
Total.....		55	30	18	8	1	1	1	114

TABLE 9  
*Passages of the McCoy Strain of Plasmodium vivax*

YEAR	PASSAGES	PATIENTS			TERMINATION SPONTANEOUS					TERMINATION INDUCED				
		Primary inoculation	Takes	Clinical attacks	Primary attack only	Secondary attacks				Primary attack only	Secondary attacks			
						1	2	3+	Total		1	2	3+	Total
1931	1-4	33	29	27	5	4	2	1	7	5	8	2		10
1932	5-9	56	47	45	12	8	3	1	12	9	5	6	1	12
1933	10-14	37	28	27	10	7	1	2	10	3	2	1	1	4
1934	15-20	32	26	24	10	3	1	1	5	4	3	1	1	5
1935	21-25	31	26	25	15	5	1	1	7	1	1		1	2
1936	26-31	46	35	34	14	7	4	1	12	4	1	3		4
1937	32-37	53	37	36	16	4	5		9	5	3		3	6
1938	38-43	38	36	35	14	8	4	1	13	2	2	4		6
1939	44-49	38	29	28	18	5	1		6	2	1	1		2
1940	50-56	38	33	32	17	5	2	1	8	5			2	2
1941	57-60	20	18	18	8	1			1	6	1		2	3
Total.....		422	344	331	139	57	24	9	90	46	27	18	11	56

TABLE 10  
*McCoy Strain: Relation of Season of Inoculation to Secondary Attacks*

SEASON	TERMINATION SPONTANEOUS					TERMINATION INDUCED				
	Primary attack only	Secondary attacks				Primary attack only	Secondary attacks			
		1	2	3+	Total		1	2	3+	Total
Winter.....	25	15	7		22	11	8	6	1	15
Spring.....	39	16	2		18	7	4	3	5	12
Summer.....	34	12	7	7	26	17	7	4	3	14
Autumn.....	41	14	8	2	24	11	8	5	2	15
Total.....	139	57	24	9	90	46	27	18	11	56

## ARTIFICIALLY INDUCED VIVAX MALARIA

In contrast to these observations, it may be recalled that in the series of artificially induced vivax infections observed up to 1939, the mean duration of the primary attack in 37 patients was  $16.85 \pm 1.80$  days, with a maximum of 64 days (5). Secondary clinical activity occurred in  $37.04 \pm 9.29$  per cent of these. Strikingly, however, the remissions were of short duration, all secondary activity arising within 8 weeks of the termination of the primary attack. It should furthermore be recalled that even the slightest degree of therapeutic interference with the artificially induced vivax infections, frequently effected a termination of the infection, rather than a remission (6).

## SUMMARY

In a series of 375 white patients naturally inoculated with vivax malaria, renewed or secondary clinical activity was more frequently observed following therapeutic interference with the primary attack, than when the latter terminated spontaneously. Clinical reactivation was not noted subsequent to attacks which came to spontaneous termination after 55 days of clinical activity, or in those which attained 48 days duration before interruption. Patients receiving adequate therapy subsequent to a spontaneous termination of their primary attack, did not experience subsequent secondary attacks; but of those not treated 46.5 per cent had further activity. Therapeutic interruption without further therapy resulted in secondary attacks in 73 per cent of the patients, but with adequate therapy in not over 25 per cent.

There is not evident any tendency to effect therapeutic interference at one rather than another period of the illness. It does not appear that the period at which therapy was initiated influenced the likelihood of bringing about a termination. Full therapy (i.e., 14 grams of quinine or 1.5 grams of atabrine) terminated the infection in 77.8 per cent of the patients, while smaller amounts only effected this result in 29.0 per cent. When the effect of interference was the production of a remission rather than termination, small amounts of parasitocidal drugs produced remissions not exceeding four weeks; but after full therapy in seven out of 10 instances they exceeded 180 days in duration. The duration of the remission tends to vary directly with the amount of the particular drug administered.

Spontaneous remissions are usually not over 2 weeks in duration, and none was observed exceeding 61 days. Second or third remissions do not materially differ from the first. Induced remissions (usually the first) often exceed the spontaneous in duration, but the subsequent secondary clinical activity does not materially differ in duration. Remissions varying from 67 to 282 days in duration accounted for 22.8 per cent of those experienced by the interrupted group. The shorter remissions in the latter group present a frequency incidence similar to those observed in the spontaneous group, while the longer are peculiar to the latter group alone. It is suggested that remissions whose length is in odd-numbered days are terminated by the reactivation of the parasite brood, the suppression of which produced it. The remissions which lasted 61 days or less, more frequently were of an odd number of days in duration, while in those exceeding this limit the duration was more often of an even number of days.

Some of the attacks which came to spontaneous termination were in general milder than those which were interrupted, an opinion based on the lower proportion of the former which were quotidian throughout, and the larger proportion of quotidian attacks which became tertian and of those which were tertian throughout. However on reactivation of the interrupted attacks, a larger proportion of the secondaries were tertian than were observed in the group the primary attacks of which terminated spontaneously.

The initiation of secondary clinical activity exhibits two frequency distributions. One distribution range is common to both the spontaneous and interrupted groups. In the former its maximum extension is marked by the 132nd day following inoculation, in the latter group it extends to the 174th day. The second frequency range begins about the 204th day, and our observations carry it on to the 415th day; it has as yet only been observed in the interrupted group. While it is not unreasonable to ascribe the activity in the first range to the primary parasitemia, difficulties arise in the case of the second range. It is noteworthy that the onsets of conspicuous examples of protracted incubation fall in the second range.

Most of the infections analyzed were induced with the McCoy strain, which during the period considered experienced 60 uninterrupted mosquito-human passages. No changes were observed in its characteristics during this time.

## DISCUSSION

The continuous clinical activity initiated at the end of the incubation period in vivax malaria is logically denominated the primary attack, and is clearly a manifestation of the primary parasitemia. When a continuous series of paroxysms ceases without later resumption of clinical activity, the duration of the primary attack is clearly marked. When the continuity is interrupted by the occurrence of spontaneous or induced remissions of short and variable duration, definition of the termination point is difficult, as most distinctions have been made on a purely arbitrary basis. Our present criterion of a minimal remission of 5 days is obviously inadequate.

The circumstance that the onsets of the renewals of clinical activity exhibit two frequency distributions, one common to secondary activity arising after either spontaneous or induced termination of what is obviously a part of the primary parasitemia, the other observed only after therapeutic interference, indicates that we are probably dealing with two dissimilar groups. The first has been observed to extend as long as 174 days from inoculation, and represents, we believe, activity of the primary parasitemia. Remissions occurring in this range of time do not mark the presumptive termination of the primary parasitemia, and hence of the primary attack. On the other hand, activity

arising after the 204th day probably is not related to the primary parasitemia, and hence can be correctly regarded as secondary. We suggest that the term recrudescence be limited in its application to the former, and the term relapse to the latter.

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# OBSERVATIONS ON THE POSSIBLE USEFULNESS OF THE COMPLEMENT-FIXATION TEST IN THE EARLY DIAGNOSIS OF YELLOW FEVER\*

ALINA PERLOWAGORA, AND EDWIN H. LENNETTE†

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In a previous communication (1) a complement-fixation test for the diagnosis of yellow fever virus infections in man and experimental animals was described, and its usefulness for this purpose was demonstrated by the high degree of correlation found to exist between the results obtained by this method and those obtained with the neutralization test. In certain directions, however, the usefulness of both tests is limited by the relatively late appearance of the antibodies these tests were devised to detect, and a diagnostic procedure applicable to the early stages of yellow fever is frequently desired.

It is the object of this report to present data which, in agreement with the original observations of Davis (2), indicate that the complement-fixation test, when employed to demonstrate the presence or absence of the specific complement-fixing antigen in sera taken during the acute phase of suspected yellow fever infection, may meet this need.

While infectious monkey serum was used as a source of complement-fixing antigen by early workers on yellow fever, there is in the literature no uniformity of opinion as to the time and regularity of appearance of the antigen in the blood. Thus, Aragão (3), using as antigen human sera taken on the first or second day of illness was unable to demonstrate complement-fixing antibodies in convalescent human sera. Frobisher (4) reported that fixation was weak and irregular when monkey serum antigens were used. Davis (2), however, was able to demonstrate the presence of antigenic substances in the serum of 40 of 43 monkeys bled from one to four days after inoculation of virus (two monkeys which furnished the negative specimens survived, the third died). Because of the

regularity with which the antigen appeared in the blood, Davis suggested that the complement-fixation test might be applied to the early diagnosis of yellow fever. Frobisher subsequently reported that while pooled early-fever sera constituted a satisfactory antigen (5), the use of individual serum specimens as antigen was accompanied by a high proportion of falsely positive or negative results (6).

## MATERIALS AND METHODS

*Animals.* Monkeys (*Macaca mulatta*) and Brazilian marmosets (*Callithrix jacchus*) were inoculated with virus and bled at intervals thereafter; in the majority of cases the serum specimens, in addition to being examined for antigenic properties, were inoculated into mice either to determine whether virus was present or to ascertain the virus content.

To test for the presence of virus, a small aliquot of serum was removed from the clot within several hours after the blood sample was drawn, and was injected intracerebrally in 0.03 ml. amounts into a group of six mice from 17 to 27 days of age. For titration purposes, serial 10-fold dilutions of the serum were made in physiological salt solution containing 10 per cent of normal human or monkey serum; each dilution was inoculated intracerebrally into six mice, in doses of 0.03 ml. per animal. The mice were checked daily to record deaths, and the virus titer was calculated according to the 50 per cent mortality end point method of Reed and Muench (7).

The remainder of the serum was stored in the refrigerator until sufficient specimens accumulated to warrant setting up the fixation tests.

Temperature readings were taken twice daily on all the monkeys and on most of the marmosets; since marmosets, as previously pointed out by Laemmert (8), only infrequently react to infection with yellow fever virus with a temperature elevation, daily temperature readings were made chiefly in those experiments in which positive information as to the presence or absence of fever was required for correlation with other data.

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† From the Serviço de Estudos e Pesquisas sobre a Febre Amarela, Rio de Janeiro, Brazil.

*Serum antigens.* Where several specimens of blood were taken from an animal, the sera were all examined for antigenicity in the same complement-fixation test. Prior to use all sera were inactivated at 60°C. for 30 minutes; the sera were diluted 1:5 or 1:10 with physiological saline to avoid coagulation or precipitation which might occur at this temperature with undiluted sera.

*Liver antigens.* To reduce to a minimum the nonspecific reactions reported to occur with liver antigens (5, 9), the method of Casals and Palacios (10) for the preparation of brain antigens was followed.

Livers were removed from monkeys or marmosets found dead, or killed when moribund; a portion was coarsely minced with scissors, weighed, triturated in a mortar with alundum and made into a 10 per cent suspension by the gradual addition of 0.85 per cent salt solution. This suspension was kept in the icebox overnight and then centrifuged horizontally for 30 minutes at 2500 r.p.m. in an International electric centrifuge. The supernatant was drawn off, frozen and thawed five times in a mixture of solid carbon dioxide and alcohol, which resulted in the precipitation of a small amount of amorphous material, and then spun at 3000 r.p.m. for one hour in an International centrifuge equipped with an angle head. The supernatant from this run was passed through a Seitz EK pad; and the filtrate, after the addition of merthiolate to a final concentration of 1:10,000, constituted the antigen.

*Immune and normal test sera.* The antigenicity of liver suspensions and of acute-phase sera from infected monkeys and marmosets was tested by the use of pooled rhesus immune serum of known complement-fixing potency; several pools were required during the course of the work. Pooled normal rhesus serum, proved free of complement-fixing antibodies, was included in each test as a control.

Both the immune and the normal serum pools were always used in a 1:4 dilution and were inactivated at 60°C. for 30 minutes before use.

*Complement.* Fresh guinea pig serum was used for complement. Before each test the complement, diluted 1:10 and 1:20 in saline, was titrated in the presence of 0.2 ml. of the highest antigen concentration (1:5 or 1:10 dilution in case of serum, undiluted in case of liver suspensions) to be used, and subsequently was diluted so that 0.2 ml. represented two full units, the amount used in the test.

*Sensitized sheep cells.* Sheep red blood cells were washed repeatedly with 0.85 per cent salt solution until the supernatant fluid was perfectly clear and colorless. The packed cells obtained by horizontal centrifugation at 2500-3000 r.p.m. for 15 minutes were resuspended in saline to make a 3 per cent suspension and were sensitized by mixing the suspension with an equal volume of rabbit anti-sheep hemolysin diluted to contain three hemolytic units per 0.25 ml. The mixture was kept at room temperature for 30 minutes before being used to titrate complement.

*Complement-fixation tests.* These were conducted according to the technique previously described (1). Serial twofold dilutions (beginning with 1:5 or 1:10 in the case of serum) were made of each antigen specimen to be tested. To 0.2 ml. of each antigen dilution were added 0.2 ml. of complement dilution followed by 0.2 ml. of 1:4 immune or normal serum. After a primary incubation period of one hour at 37°C. followed by 18 to 20 hours at 4°C., 0.5 ml. of sensitized sheep cell suspension was added, and the mixtures were further incubated at 37°C. for 30 minutes. At the end of the secondary incubation the extent of fixation was determined and recorded as ++++ when complete inhibition of hemolysis had occurred and as - when complete hemolysis had occurred; partial inhibition of hemolysis was recorded as +, ++ and +++ according to the amount. The fixation end point was taken as the highest original dilution of antigen which gave at least +++ fixation. Appropriate controls on the hemolytic system and on the anticomplementary activity of the reagents were included in each test.

#### EXPERIMENTAL

*Antigenic activity of monkey and marmoset sera.* Preliminary investigations were made to determine whether complement-fixing antigen appeared in the blood of monkeys and marmosets and to ascertain whether the test adopted was suitable for the demonstration of the antigen. For the latter purpose it was necessary to choose a period during the infection when the antigen might be considered as most likely to be present; in view of the uncertainty in the literature regarding the first appearance of the antigen, it was decided to take serum specimens as close as possible to the day of death of the animal. As will be seen from table 1, the test was of adequate sensitivity for the detection of the antigen.

The results obtained with six monkeys and 17

marmosets are shown in table 1, in which the animals are listed by virus strain and according to the day of bleeding in relation to the day of death. It will be observed that complement-fixing antigen was present without exception in the blood of all the animals at or just prior to death and in the case of monkey no. 6 was present four days before the death of the animal.

this point, as well as on the relation of fever to the time of appearance of the antigen, the experiments described in the following section were done.

*Time of appearance of complement-fixing antigen and its relation to the virus.* Twelve monkeys and 14 marmosets inoculated with various strains of virus were subjected to frequent bleedings, and the sera were examined for their content of virus

TABLE 1

*Occurrence of complement-fixing antigen in sera of monkeys and marmosets infected with yellow fever virus*

ANIMAL	VIRUS STRAIN INOCULATED	RESULT OF INOCULATION		EXAMINATION OF POSTINOCULATION SERUM		
		Fever	Death	Serum taken	Virus titer	C.-F. antigen
Monkey		day	day	day		
1	Asibi	3rd	3rd	3rd	$10^{-6.5}$	Present
2	Asibi	2nd, 3rd	4th	3rd	*	Present
3	Asibi	3rd, 4th	5th	5th	$10^{-3.6}$	Present
4	Asibi	5th, 6th	7th	7th	†	Present
3	O. C.	2nd, 5th	5th	5th	$10^{-6.8}$	Present
6	O. C.	2nd thru 8th	9th	5th	‡	Present
Marmoset						
1	O. C.		4th	4th	$10^{-7.6}$	Present
2	O. C.	None	5th	4th	$>10^{-7}$	Present
3	O. C.		5th	4th	$10^{-7.0}$	Present
4	O. C.	4th	5th	5th	$10^{-6.7}$	Present
5	O. C.		5th	5th	$10^{-6.0}$	Present
6	O. C.		5th	5th	$10^{-8.0}$	Present
7	O. C.	4th, 5th	6th	4th	*	Present
8	O. C.	None	6th	5th	$10^{-6.3}$	Present
9	O. C.	5th	6th	6th	*	Present
10	O. C.		6th	6th	$10^{-9.0}$	Present
11	O. C.		6th	6th	$>10^{-9}$	Present
12	O. C.		7th	5th	$10^{-7.4}$	Present
13	O. C.		7th	6th	$10^{-9.0}$	Present
14	O. C.		11th	11th	$10^{-8.0}$	Present
15	A.C.-Bol.		4th	3rd	‡	Present
16	A.C.-Bol.		6th	4th	‡	Present
17	A.C.-Bol.		6th	4th	‡	Present

\* Serum not tested for virus.

† Virus not detected in undiluted serum.

‡ Serum not titrated; virus present in undiluted specimen.

With the exception of monkey no. 4, virus was demonstrable, or present in high titer, at the same time as the antigen in the blood of all monkeys and marmosets tested for both. While virus and antigen occur simultaneously in the serum in the terminal stages of the infection, this does not necessarily indicate that the complement-fixing activity of the serum is attributable to the virus *per se*. In an attempt to obtain information on

and of antigen. Owing to their small size and frailness, repeated daily bleeding of marmosets is attended by a high mortality rate; for this reason it was considered prudent to bleed a part of the animals during the early phase of the infection and the remainder during the later phases. Eight of the 12 monkeys were bled daily up to the time of death. The remaining four monkeys represented animals used for other purposes in this



[illegible]





[illegible]

\* Serum not tested for virus.

† Virus not detected in undiluted serum.

† Subsequently died of tuberculosis.

‡ Serum not titrated; virus present in undiluted specimen.

laboratory and hence a complete series of serum specimens was not available; these animals were included because it was of interest to determine whether the antigen appears in the blood of monkeys which survive infection with the virus. All serum specimens were titrated for antigen content, and wherever possible or feasible the freshly drawn sera were titrated for virus content.

The results are given in table 2. It will be noted that in five of the eight monkeys which succumbed to infection the antigen was first detected in the blood on the third day after inoculation of virus. In one animal (monkey no. 12) the antigen appeared as early as the second postinoculation day and in the remaining two (nos. 7 and 14) it did not appear until the fourth day.

The early appearance of the antigen in the circulation was confirmed in marmosets. Table 2 shows that in four marmosets in which the serum was examined from the second postinoculation day on, the antigen first appeared on the third day, and in six animals in which sera were examined from the third day on, the antigen was already present on the third day in five. In several instances (marmosets nos. 26, 27, 28) the antigen was not demonstrable in the blood until the fifth or sixth day.

On the other hand, in the four monkeys and one marmoset which survived infection, the antigen was not detected in any of the serum specimens tested; the possibility exists, however, that its presence may have been missed by failure to examine the blood daily over a sufficiently long period of time.

As far as can be judged from the present limited experience, the time of appearance of the antigen in the blood was roughly dependent on the length of the interval between inoculation and death of the animal; when this period was of 3 to 5 days duration, the antigen usually appeared on the third day; when longer, the antigen appeared from one to three days later, i.e., between the fourth and the sixth days.

If the febrile reaction is in any way related to the appearance of the antigen, it is not readily apparent from the data in table 2. Thus, despite the absence of a febrile reaction in the 11 marmosets whose temperatures were taken twice daily up to the time of death, the antigen occurred in the blood of all. Of the eight monkeys in which the infection terminated fatally, the appearance of the antigen coincided in five instances with the onset of fever, in one the antigen appeared on the

day following a rise in temperature and in two the antigen appeared in the absence of fever.

It will also be noted that although the appearance of the antigen tended to coincide roughly with an increase in circulating virus toward the maximum reached, this may be more apparent than real, since the antigen was not detected, despite the presence of relatively large amounts of circulating virus, in three monkeys and a marmoset which survived.

From the lack of parallelism between the virus and antigen content of the sera, it appears that antigenicity is associated with a factor which is distinct from the virus, a conclusion previously reached by Hughes (11) in his study of the yellow fever precipitinogen. Hughes reported that the amount of circulating precipitinogen (and, by inferences, the amount of circulating complement-fixing antigen) varied roughly with the severity of the infection and was thus a reflection of the amount of tissue damage provoked by the virus. According to this author, the precipitinogen and complement-fixing antigen may possibly represent products of cellular injury caused by the virus. The febrile response to inoculation of the virus may conceivably be taken to indicate that sufficient tissue damage has occurred to elicit the presence of these substances in the circulation, but the absence of a febrile reaction does not necessarily rule out the occurrence of such an event.

Monkeys nos. 15-18 inclusive and marmoset no. 31 may be taken as instances in which the hosts' reaction to the virus was minimal, since inoculation of the virus did not result in the appearance of detectable amounts of antigen in the serum specimens tested, and the elevations in temperature which occurred were questionable (monkey no. 15) or nonspecific (monkeys nos. 17 and 18, which died of tuberculosis). Nevertheless, some reaction must have been elicited since serum specimens taken two to three weeks after inoculation of the virus contained both neutralizing and complement-fixing antibodies. The results are at variance with the findings of Hughes on the precipitinogen (11), but this may be due to the greater sensitivity of the complement-fixation test.

*Correlation between serologic and histologic diagnosis of yellow fever.* If the complement-fixing antigen represents a foreign protein formed and liberated from tissues attacked by the virus, it would be expected that the liver, which is almost invariably, and frequently severely, involved, would constitute one of the main sites of origin of

the antigen. The results of the complement-fixation tests with sera were therefore checked by examining the livers of the corresponding animals for the presence of antigen and of lesions.<sup>1</sup>

The data on 14 monkeys and 23 marmosets are summarized in table 3. In 11 of the monkeys the antigen was detected both in the blood during the course of the infection and in the liver at death;<sup>2</sup> histologic examination of the liver revealed the presence of lesions of the type associated with experimental yellow fever. In the remaining three monkeys, the antigen was demonstrable in the blood, but not in the liver. A histologic diagnosis of yellow fever could not be made on these three

TABLE 3

*Comparison of the results obtained by the serologic and the histologic methods of diagnosis of yellow fever*

NUMBER OF ANIMALS TESTED	DIAGNOSIS BASED ON PRESENCE OF C.-F. ANTIGEN IN				DIAGNOSIS BASED ON HISTOLOGIC EXAMINATION OF LIVER		
	Serum		Liver		Pos.	Neg.	Questionable pos.
	Pos.	Neg.	Pos.	Neg.			
Monkeys 14	11 3		11 3		11	3	
Marmosets 23	20 3		20 3		16	2 3	2

livers; two showed only slight fatty changes, and no necrosis or inclusion bodies, and the third showed necrotic changes considered as atypical of yellow fever.

As in the case of the monkeys, the antigen was found in the blood of all the marmosets. In 20 of the 23 animals the antigen was also present in the liver, although a diagnosis of yellow fever could not be made on four of the livers, which showed only evidence of fatty degeneration or a

<sup>1</sup> For histologic examination, small portions of liver were fixed in 10 per cent formol-saline and embedded in paraffin; the cut sections were stained with hematoxylin and eosin.

<sup>2</sup> It should be noted here that in all cases in which the antigen was found in the liver, it was also found in the blood, but not vice versa. Since no attempts were made to free the liver of blood before testing for the presence of antigen, the possibility exists that the antigenicity of the liver preparations was due to the residual blood within the organ and not to the hepatic issue itself.

few necrotic cells. In the remaining three animals, the liver contained neither the antigen nor lesions which would permit of a positive diagnosis of yellow fever (one specimen showed only a few foci of necrosis considered as atypical and the other two showed fatty degeneration; no necrosis or inclusion bodies were noted).

Although a diagnosis of yellow fever could not be established from histologic examination of the liver of the 10 animals referred to above, other evidence aside from the presence of antigen in the blood indicated that infection with the virus had occurred. Thus, two monkeys and one marmoset reacted to inoculation with an abrupt rise in temperature on the second to fifth days, and all 10 animals became prostrate and showed a terminal drop in body temperature. Circulating virus was present in all, occasionally in high titer.

It appears from table 3 that the demonstration of complement-fixing antigen in the serum of monkeys and marmosets constitutes a more accurate diagnostic means than does histologic examination of the liver; until a wider experience is gained, however, it is impossible to state whether or not the difference observed in the results of the two methods is a true one. Six of the 10 animals in which the histologic examination of the liver resulted in a diagnosis of "negative" or "questionable positive" were killed when believed to be in a moribund state and hence may have been killed before typical lesions had an opportunity to appear. On the other hand, the majority of the 27 positive animals were killed under essentially similar conditions, and no difficulty was encountered in establishing a histologic diagnosis of yellow fever.

#### COMMENT AND SUMMARY

By the usual methods, a diagnosis of yellow fever in man early during the course of the illness is impossible, since these are based on the isolation and identification of the specific virus or on the demonstration that specific antibodies appear as a result of the infection. Davis (2) as early as 1931, however, showed that the complement-fixing antigen appears with regularity in the blood of monkeys succumbing to infection with yellow fever virus, and Hughes (11) in 1933 made a similar observation with regard to the precipitinogen. Both authors suggested that the occurrence of the antigens they were dealing with might afford a means for arriving at a positive diagnosis of yellow fever in the acute phase of the disease. No further work appears to have been done on this subject,

however, and because of the practical considerations involved a reinvestigation of the matter was considered worthwhile.

The complement-fixation test was chosen in preference to the precipitin test since in our experience it proved to be the more reliable, and the presence of the complement-fixing antigen could be readily demonstrated in sera apparently devoid of the precipitinogen.

Eighteen monkeys (*Macaca mulatta*) and 31 marmosets (*Callithrix jacchus*) were employed in the present investigation. These animals were inoculated with the classic Asibi strain of yellow fever virus or with the O.C., A.C.-Bol., or Volcanes jungle strains of the virus, and their sera were tested for the presence of complement-fixing antigen on one or more occasions after inoculation of the virus. The complement-fixing antigen was present in the blood of all those animals which died or were sacrificed when moribund, but was not detected in the blood of those which survived infection and subsequently developed complement-fixing as well as neutralizing antibodies.

The lack of correlation between the virus content and the antigen content of a serum indicates that complement-fixing antigenicity must be referred not to the virus *per se* but to some substance resulting from the reaction provoked in the host's tissues by the virus. A specific febrile response following inoculation of virus indicates only that the reaction to the parasite is of sufficient magnitude to evoke the appearance of the antigen. The absence of a postinfection temperature rise does not necessarily imply the converse, however, since the antigen has been detected both in monkeys and in marmosets which succumbed to infection without showing a febrile reaction.

At least two obvious explanations exist for the failure to demonstrate antigen in the blood of those few animals which survived infection; either (a) the serum specimens tested were not taken at the appropriate intervals, or (b) the antigen was present in concentrations too small to be detected by the technique used. Experiments now in progress indicate that the latter explanation is probably the correct one, and it would seem, therefore, that the antigen appears in demonstrable amounts chiefly (and perhaps solely) in the blood of those animals in which the disease may be expected to terminate fatally.

In 10 of 37 animals a post-mortem diagnosis of yellow fever virus infection could not be made by histologic examination of the liver, although it

was made during life by examination of the serum for complement-fixing antigen. This suggests that the serologic test may therefore constitute a more accurate diagnostic method than does microscopic examination of the liver, although a broader experience is necessary before any unqualified statement is possible as to the greater accuracy of one method over the other.

Opportunity to examine human yellow fever sera has not been available, but if the antigen occurs in the blood of man also it should be possible to use the complement-fixation test for the early diagnosis of the disease in this host, provided that the method is applicable to all degrees of infection, and is not limited to severe or fatal cases.

#### ACKNOWLEDGEMENT

We are indebted to Dr. M. Pará for examination of the histologic preparations and interpretation of the pathologic changes.

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# YELLOW FEVER CONTROL DURING THE WAR<sup>1</sup>

C. L. WILLIAMS<sup>2</sup>

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Among war-time problems is the potential danger of introduction of yellow fever into the southern United States. This threat, made serious by the advent of international air travel, has been greatly increased by the dislocations and confusion of war. The Public Health Service, which is charged with the exclusion of quarantinable diseases, has become so much concerned that it has gone to some lengths to organize a workable and effective control program, ready for immediate operation on short notice.

## QUARANTINE MEASURES

Since the recession of yellow fever into the vastnesses of the interior of South America and Africa, ship transfer of the disease has become unlikely, and quarantine measures directed against ships have been greatly reduced. Airplane traffic, however, is another story, and offers a real danger of introduction of the disease.

There are two avenues through which infection may be brought. One is an infected mosquito that may be carried in the cabin of an airplane coming from infected areas. The other is a passenger who has been infected, and is still in the incubation period, but shows no symptoms. The first of these dangers is combated by a very widespread system of spraying intercontinental and international aircraft with insecticide solutions before departure, while in flight, and on arrival. The responsibility for doing this with commercial aircraft lies on the U. S. Public Health Service through its Foreign Quarantine Division. Military aircraft come under the control of the Army and Navy, which have invited the cooperation of the Public Health Service in instituting adequate anti-mosquito measures.

The more real danger is introduction of infected passengers still in the incubation period. Against this, two particular measures are in operation—the more positive is immunization against yellow

fever of all military personnel sent to infected areas, and most civilians going from this country to such localities. There are, however, some non-immunized persons that arrive in this country from infected areas within the incubation period. Several years ago, it was determined that it was not feasible to quarantine airplane passengers and, in lieu thereof, for some years there has been in operation a system of surveillance whereby possibly infected persons are kept under observation by State, municipal and County Health Officers wherever they may go until the possible infective period has passed.

Briefly, the details of surveillance are these: The quarantine officer on inspection determines the persons who must be kept under surveillance. He requires of them a statement of their destination or destinations and either writes or wires the health officer concerned, requesting surveillance until a specified date—this being nine days following the last possible exposure to yellow fever. Nine days is used to cover six days of incubation and three days of infectivity to mosquitos. Copies of such notifications to health officers are sent to the State Health Officer in every instance. It will be noted that this system involves the cooperation of State, municipal and County Health Officers. At first it was somewhat haphazard, but has steadily improved. Recent check surveys indicate about seventy-five per cent effectiveness.

It will be recognized at once that the purpose of surveillance is not to prevent the entrance of yellow fever into the country, but to reveal immediately its introduction. Obviously, for such early notice to be of value, we must be prepared to institute control measures at once.

## CONTROL MEASURES

Upon the advent of war, the U. S. Public Health Service realized that the danger of introduction of yellow fever would rapidly increase, and at once instituted measures to train personnel for its control in the event a case was reported. Upon the establishment of the field office of Malaria Control in War Areas, the yellow fever program was placed in its hands, principally because it pre-

<sup>1</sup> Read at the Annual Meeting of the American Society of Tropical Medicine, at Cincinnati, Ohio, November 15-18, 1943.

<sup>2</sup> Medical Director, U. S. Public Health Service.

sessed the necessary organization, specifically trained personnel, and specialized material and supplies. That office, in cooperation with the Director of U. S. Public Health Service District No. 4, established a definite plan of procedure which it is now prepared to put into operation within twenty-four hours in any locality in the usually accepted infectible area.

The control program is based upon four specific operations. First control of cases and contacts; second, destruction of adult *Aedes aegypti*; third, immunization of possibly exposed persons; fourth, control of *Aedes aegypti* breeding.

The first of these operations is clearly a function of the local health department, and it is expected would be carried out by that organization. It would include immediate isolation of cases and contacts in mosquito-free and mosquito-protected premises. The establishment of *aegypti* free detention camps should be necessary only in case infection had become widespread before discovery.

To destroy adult *Aedes aegypti*, the Malaria field office has two trucks completely equipped and ready to move to any place in the country. These are ready-packed with an adequate supply of pyrethrum solution and pressure and power sprayers of various types. Personnel to man them is already designated. Immediately on notification, they would be manned and sent overland to the infected area, and would there be available for disinsectization of all premises. The pyrethrum spray to be used is a much stronger solution than is contained in commercial insecticides usually sold on the retail market, and is exceedingly effective against mosquitos, killing one hundred per cent of them in enclosed areas through the application of quite small amounts of the material. The pressure and power sprayers available are very efficient pieces of apparatus so that a relatively small force of men can disinsect quite a number of houses during the course of the day. Pyrethrum sprays are practically non-toxic to human beings, and their use in destroying mosquitos does not require any careful sealing of premises.

For purposes of possible yellow fever control the Public Health Service maintains at its laboratory in Hamilton, Montana a stock of one half million or more doses of yellow fever vaccine. A telegram or long-distance telephone call would have this vaccine dispatched in adequate quantities by airplane within a few hours. The plan

of utilization is to distribute the vaccine to private practitioners for use with their own patients, and to employ physicians to set up vaccination stations where the public at large would be immunized. It seems unlikely, in view of the fear of yellow fever that one might expect, that it would be necessary to institute any enforced vaccination campaign. The vaccine manufactured by the Public Health Service does not contain human serum and its use has not been attended with the development of outbreaks of jaundice.

The Malaria field office as part of its regular work is carrying out *Aedes aegypti* control in a number of seaports in the southern states. The personnel engaged numbers something over one hundred trained workers. In case yellow fever actually broke out, this personnel already equipped would, within a few hours, be enroute to the infected area, and within the course of a day or two would be at work on an adult and breeding control campaign.

Since *Aedes aegypti* is a domestic mosquito, very definite immediate reduction of breeding may be secured through public appeal. The material for this is already prepared and would be made immediately available to newspapers, radio stations, and public speakers. Where available, the Office of Civilian Defense would doubtless be happy to utilize its very efficient block leader and air raid warden organization.

#### ADMINISTRATION

The yellow fever control measures that would be put into operation are broadly outlined above. The details of operation in the field cannot, in this short paper, be described, but available for this work are trained workers who, through their training, carry the details in mind. The administrative procedures of employment, purchasing, reporting etc. would be handled through an expansion of these functions already in operation by the Malaria field office. The only administrative details offering any material difficulty are those involving setting the program rapidly into operation. These for the most part have been determined upon and even the personnel who will give the orders designated. Mostly, they will be accomplished by long-distance telephone, and the places and persons to whom such telephone calls should be made have been designated, and are on record.

## RELATIONS WITH THE STATES

The public Health Service, of course, has always worked in cooperation with the State Health Departments, and no other method of procedure is contemplated for yellow fever control. The State Health Officers, in infectible territory have already been consulted and are informed that the assistance here outlined is available to them immediately on request. They have all expressed themselves as happy to know that this very considerable organization can be secured on a moment's notice. In actual operation, it is expected

that it would proceed very much as does Malaria Control in War Areas, through the State health organizations which would impose the authority of the State utilizing the technical advice and guidance of the expert personnel supplied by the Public Health Service.

We hope we may go through this war without the introduction of yellow fever. If it should occur, however, we will not be found unprepared and we believe that we have taken reasonable steps to insure its immediate detection and rapid control.





# A CONSIDERATION OF CERTAIN PROBLEMS PRESENTED BY A CASE OF STRONGYLOIDIASIS

EDDY D. PALMER<sup>1</sup>

University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

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A case of *Strongyloides stercoralis* infection was recently diagnosed in northern New York State. Although that fact is not particularly remarkable in itself, the case presents certain problems which seem to justify discussion.

## CASE REPORT

K. K., #97270, 63 year old male white Polish junkyard worker, admitted to the Rochester Municipal Hospital on May 27, 1943 and died on the fifteenth day thereafter. Chief complaint was "stomach trouble and diarrhea" of two weeks duration. Present illness began suddenly with sharp nonradiating periumbilical pain and watery diarrhea without gross blood or pus seven to eight times a day; symptoms persisted up to the time of admission. One week before admission the patient began to vomit recently ingested food without gross blood or feces; he then stopped taking food, and for about six days prior to admission took little but ethyl alcohol. For several days he had had generalized body aching. There had been no chest pain, cough or expectoration.

The patient lived for 33 years of his life in Poland, then immigrated directly to Rochester, New York, and had not left this city since his arrival 30 years ago. Nine years ago he was examined at this hospital for acute intestinal perforation. Much cloudy fluid was found throughout the abdominal and pelvic cavities, and a large very hard mass grossly resembling a carcinoma was found involving the ascending colon and hepatic flexure; there were large hard nodes scattered through the mesentery, and the omentum was adherent to the mass. No point of perforation was found, and the mass could not be mobilized. An ileotransverse colostomy was done and tissue taken for biopsy. *B. coli* was cultured from the abdominal fluid, and the biopsy showed a hard yellow-white tissue which was found to be organizing connective tissue with many round cells and fewer polymorphs. The postoperative course was uneventful, and apparently the patient had remained well until the present episode.

Physical examination showed an acutely ill, dehydrated, well developed middle aged male, temperature 38.8°C., pulse 84, respirations 40, and blood pressure 130/85. The skin was clear without petechiae or cy-

anosis. The fingers were clubbed. The thorax showed good expansion but intercostal retraction laterally. The lungs presented no abnormal signs and abdomen was flat with an old right upper rectus scar; there was moderate voluntary spasm throughout. The spleen was felt one fingerbreadth below the costal margin. No abdominal masses, point tenderness or fluid was detected. Rectal examination revealed no masses or tenderness or gross blood on the examining finger.

Admission laboratory studies showed negative Wassermann and Kahn tests, leukocyte count of 6,400, erythrocyte count of 4.0 millions, and hemoglobin of 13.0 grams. The red cells appeared normal in size, shape and color, the number of platelets appeared adequate, and a differential count demonstrated 67% neutrophils, 22% lymphocytes, 8% monocytes, 2% eosinophils, and 1% basophils. The urine showed a trace of albumin. A glove fecal fragment was dark brown without gross blood but gave a positive guaiac reaction, and direct microscopical examination revealed no evidence of intestinal parasitosis. The blood nonprotein nitrogen was 28 milligrams, albumin 2.9 grams, globulin 1.7 grams, chlorides 554 milligrams, and carbon dioxide combining power 44 volumes per 100 cc. The admission blood culture, Widal agglutinations with H and O antigens, and stool culture for pathogens were negative. A concentrate of fasting gastric contents on the tenth day was negative for tubercle bacilli. A stool was purified for tubercle bacilli but the guinea pig died before a satisfactory examination could be made.

A chest plate taken on the day of admission showed a large calcified plaque on the left leaf of the diaphragm and numerous large round splenic calcifications. There were extensive calcifications at both lung roots. The lower portion of the left lung field and the whole right field showed hard linear markings with soft peribronchial densities. The plate was interpreted as revealing an extensive old infection with fibrotic change and old calcified tuberculous lesions.

The patient was placed on atropine sulfate and hydrated parenterally. The temperature remained near 39°C. throughout the hospital course. The pulse climbed slowly from 84 at admission to 160 on the day of death. The respiratory rate varied from 32 to 50; the patient never complained of dyspnea although often respirations appeared labored. Crepitant rales slowly

<sup>1</sup> Lieutenant, Medical Corps, AUS.

spread upward through the chest, and on the third day a small area of bronchial breath sounds was found in the right axilla. Dullness to percussion was found in the right axilla and at the base on the tenth day. There was no cough or sputum until the thirteenth day. The abdomen became softer, but mild diffuse tenderness persisted.

The leukocyte count remained low. On the third day it was 5,500 and on the fourth 5,000. Sulfathiazole treatment was instituted on the fourth day but discontinued after a total of 11 grams had been administered because the count dropped to 2,400. From the sixth to the twelfth days the leukocyte count varied from 4,000 to 5,200. The differential count showed no tendency toward a constant significant shift; the percentage of eosinophils varied from zero to 2% in counts of 200 cells.

Rectal tenesmus was persistent throughout the hospital course. The patient had his first bowel movement on the third day. It was brown, liquid and guaiac positive. Thereafter there were one to three liquid stools a day, all guaiac positive, and many with much mucus. Several direct microscopic examinations revealed no protozoa or helminth eggs or larvae. A zinc sulfate centrifugal flotation examination was made on a liquid stool on the sixth day, and many rhabditiform *Strongyloides stercoralis* larvae were found. No other parasites were seen.

On the seventh day the patient was started on a course of enteric-coated gentian violet medicinal, 0.06 gram t.i.d. before meals. Larvae were abundant in direct stool examinations on the ninth day when the gentian violet first appeared in the feces, but by the thirteenth day they were not to be found in stool concentrates.

On the twelfth day the patient appeared worse and the pulmonary lesions were obviously progressing rapidly. A chest plate showed an increase in the soft peribronchial infiltration scattered through both lung fields over that found in the admission plate. On this day 0.1 gram of gentian violet was administered intravenously in 0.5% aqueous solution.

On the thirteenth day the patient had a severe shaking chill lasting ten minutes with a temperature rise to 40.4°C. There was severe abdominal pain with the passage of much flatus and a copious liquid bowel movement. Cyanosis was moderate. The abdomen was distended and tympanitic, and there were severe generalized abdominal tenderness and rebound tenderness referred to the right. Borborygmi were audible but not marked. The leukocyte count at this time was 1,900 with 86% neutrophils showing a marked shift to the left. A chest plate revealed coalescence of the markings above the right lung root, which was increased in size over that seen in the previous day's plate; the changes were those of bronchopneumonia or of a bronchial disseminated tuberculosis. A blood

culture was negative. Sputum for the first time became available and tubercle bacilli were found.

The patient's course continued downhill, and he died on the fifteenth day after admission.

#### *Postmortem examination*

The autopsy was performed by Dr. Frank W. McKee two and one-quarter hours after death. Much pathology was found, mostly referable to a widespread tuberculous infection. Among the anatomic diagnoses were listed bilateral tuberculous bronchopneumonia, tuberculous enteritis with multiple perforations of the small bowel, acute fibrinous and tuberculous peritonitis, and other widely scattered tuberculous lesions.

Without taking space to go into detail, it may be sufficient to state that no evidence, gross or microscopic, was found indicating pulmonary or intestinal strongyloides infection. No adult or larval worms were found, and no eosinophilic tissue reaction was seen. The mucosa of the ileum was missing in several areas, and at the sites of three deep mucosal ulcerations there were punched-out perforations with rolled edges, the largest measuring 4 by 4 cm. Along the margins of the perforations there were many subserosal tubercles. The submucosa and muscularis of the ileum were edematous and heavily infiltrated with round cells and many young tubercles. There were numerous ulcerations of the cecal mucosa and these also were surrounded by tuberculous lesions. No evidence of the large bowel tumor seen at laparotomy nine years before was found.

#### NOSOGEOGRAPHY

In the attempt to reach a conclusion as to the source of the *Strongyloides* infection in this patient, it is necessary to estimate the infection's approximate duration. Faust (1931) has given helpful information on this point, stating that there is a marked eosinophilia and leukocytosis during the periods of the worms' invasion, incubation and early oviposition, but that, as the infection becomes chronic, the leukocyte count and the percentage of the circulating eosinophils decrease. Again in 1938, Faust states that, as the infection becomes chronic, there may be a leukopenia. In the case under discussion the leukocyte count varied around the lower normal limit, and the proportion of eosinophils was never found to exceed 2% and on several days no eosinophils were found in counts of 200 cells. The evaluation of the significance of these blood findings is little complicated by the coexistence of the acute disseminated pulmonary tuberculosis and tuberculous peritonitis. The high proportion of monocytes, which on two days formed 8% of the white cells,

and the low proportion of lymphocytes, which on four days was 12% or less, may indicate a blood response to the tuberculous infection. However, the white count was constantly low throughout the hospital course in spite of the expected leukocytosis which usually accompanies an acute tuberculous infection. It is fair, therefore, to say that the strongyloidiasis was probably a chronic infection.

Thus the present infection could have originated either in Poland or in Rochester, New York. These are the only possible source areas in this case. Brumpt (1936) says, "En Europe, ce parasite est loin d'être rare en Italie, en Allemagne, en Belgique, en Hollande," and, although no definite statement could be found on the subject, it is supposed that a similar situation exists in Poland. If Poland was the source area of infection, the patient had harbored worms for more than 30 years. No reported infection of such long duration has been found in the literature. Schäfer and Lodenkämper (1935) and Bodon (1941) describe cases in which the infections apparently had persisted at least 20 years.

If the present infection had, indeed, persisted for more than 30 years, it is necessary to realize that the continuation of the infection had depended on frequent autoinfections or hyperinfections or both. The fecundity of human strains of *S. stercoralis* is apparently rather short-termed, at least in experimental animals (Faust, Wells, Adams, and Beach, 1934). In summing up their work, Faust et al. (loc. cit.) conclude, "... unless internal infection is predicated, it is inconceivable that man remains infected for a period from fifteen to twenty years without outside exposure or hyperinfection... internal infection (hyperinfection) (sic) is the only satisfactory explanation for prolonged chronic strongyloidosis."

The patient under discussion gave a history of often lying on the ground during the noon hour in warm weather in the Rochester junk yard in which he worked. In view of the possibility that he might have acquired the infection in this manner and that some of his fellow workers might have done likewise, single non-refrigerated stool specimens from each of the seven yard-workers who were employed at the time the patient entered the hospital were examined by direct and zinc sulfate technics. No evidence of strongyloidiasis was found. No history of skin lesions suggestive of a

strongyloides dermatitis could be obtained from the patient.

The nosogeography of strongyloidiasis in the northern parts of the United States is imperfectly known. Hinman (1938) states that, although the incidence of the infection in the southern United States is as high as 1 to 5%, little is known of the distribution in other parts of the country. The question of autochthonicity of reported cases is, of course, the limiting factor. Autochthonous cases have been reported from New York City, eastern Tennessee, Cincinnati, and Kansas City (Faust, 1938). Ginsburg (1920) describes a case in a native-born woman who had "... lived all her life in the western part of Pennsylvania." Cadham (1933) reports the infection in a Canadian nurse who "... had been a resident of Canada since birth and for some years previous to her illness had not been outside the prairie provinces." In 1941 Bodon reported *Strongyloides* infection in an Italian man and wife who had migrated to East Rochester, New York from Italy 20 years before the infections were discovered. Both patients had lived in East Rochester since their arrival. Bodon concludes, "Both individuals undoubtedly acquired the parasite in Italy..." Faust in 1943 stated, "I think it is possible but rather unlikely that genuine cases of endemic strongyloidiasis occur in central New York State, as for example, in the Finger Lakes Region."

It appears, therefore, that no conclusion can be reached regarding the source of the infection in this patient. It would be unfortunate to discard the possibility of Rochester's being an endemic focus merely on the basis of the parasites' distribution as it is now known or on the suppositions which surround current thinking on the meteorologic factors governing the extraparasitic phases of the worm's biology in nature. Three cases of strongyloidiasis have been found in Rochester in the past three years. Certainly the question of the northern limit of the parasite's natural range invites further study.

#### DIAGNOSIS

The tendency in many laboratories to use only direct examination technics on liquid stools—as was at first done in this case—may be explained by the emphasis that has been placed on *Endamoeba histolytica* infections and the seeming necessity to prove all nonbacterial infectious diarrheas and dysenteries to be on an amebic basis. Since there

is no quick method known as yet to concentrate stools for amebic trophozoites, the forms which appear in liquid stools, they are searched for in simple direct fecal preparations. However, since several enteric helminthic parasitoses may be responsible for diarrheas and dysenteries and since the zinc sulfate centrifugal flotation technic (Faust et al., 1938, 1939) has proven successful for the concentration of *S. stercoralis* larvae and helminth eggs, to neglect concentrating liquid stools is to court missed diagnoses in these cases.

It is important, however, to bear in mind, as has been repeatedly emphasized from many quarters, that in strongyloidiasis as in other intestinal parasitoses, the repeated absence of coprologic evidence of infection does not necessarily indicate an absence of infection. Faust et al. (1934) in work on canine infections with a human strain of *S. stercoralis* found that, following the incubation period and the attaining of maximum oviposition, the egg output of the female worms gradually decreases to the zero point, the explanation being found in the local tissue reaction to the parasites. Thus the stool examination may be negative for months while many nonovulating female worms are present and producing pathology in the intestinal mucosa. Another cause for failure to demonstrate larvae in the stool, in this case in the presence of ovulating females, is found in the relative fragility of strongyloides rhabditiform larvae; Cordi and Otto (1934) in studies on the effects of various temperatures on larvae of *S. fiillleborni* found that the larvae disappear quickly from stools kept for only short periods in the ice-box.

#### PULMONARY STRONGYLOIDIASIS

*Strongyloides* pneumonitis is a well-known entity (Faust, 1939). Hinman (1937) describes two cases of *Strongyloides* bronchitis. Yoshino (1932) tells of 25 cases of *Strongyloides* enteritis, 14 of whom had a complicating bronchitis. Larvae were found in the sputums of three of the latter. Thirteen of the 25 cases died from complicating disease, mostly bronchitis. Gage (1911) tells of a patient who presented admission signs of diffuse bronchitis and lobar pneumonia of both upper lobes and who developed a cough but very little sputum; the differential count showed 3.2% eosinophils. *Strongyloides* larvae were found in the sputum. The lungs cleared rapidly although the larvae persisted in the sputum for two months.

Diarrhea developed after the patient had been observed for one week, and death followed two months after admission. Postmortem examination confirmed the diagnosis of *Strongyloides* pneumonitis and enteritis. Barlow (1915) reports a case of strongyloidiasis with signs of bronchopneumonia but emphasizes the necessity of ruling out other possible etiologic agents, such as tubercle bacilli, in such cases.

Mackie (1939) makes the statements, "... most, if not all studies of the clinical significance of the parasite (*Strongyloides stercoralis*) have been insufficiently controlled. Persons harboring the *Strongyloides* have necessarily been exposed to infection by numerous other intestinal parasites, helminthic and protozoal, to say nothing of bacterial" and "I have not seen intestinal disease which could with certainty be ascribed to *Strongyloides* infestation." The patient here presented serves as a particularly good case in point. The clinical symptoms and signs indicated severe pulmonary and intestinal pathology. *Strongyloides* larvae were found in the stools. Tuberculosis was also considered, but no tubercle bacilli could be found in gastric washings and no sputum was produced until late in the patient's course. Chest plates showed wide-spread and rapidly progressing pulmonary lesions as well as abundant evidence of ancient calcified pulmonary tuberculous lesions and splenic calcifications suggestive of old intestinal tuberculosis. Intravenous gentian violet was administered because of the possibility that the acute pulmonary lesion represented a *Strongyloides* pneumonitis, even though larvae of pulmonary origin were not recovered.

#### STRONGYLOIDIASIS COMPLICATING PULMONARY TUBERCULOSIS

Chandler (1929) emphasizes the mechanical injury to the lung produced by migrating hookworm larvae and states that pulmonary infections are more frequent and more severe in hookworm patients than in persons without hookworm infections, while Strong (1942) makes the statement that the lesions and symptoms of pulmonary irritation in *Strongyloides* infection are more severe than those noted in hookworm infection. It seems likely in the patient here under consideration that the development of the fulminating disseminated tuberculosis from an old inactive pulmonary process may have been associated with a recent migration of a significant number of

*Strongyloides* larvae through the lung in the course of their normal development. Such a series of events would suggest mass hyperinfection or auto-infection.

Martínez (1933) describes a case of intestinal strongyloidiasis complicating pulmonary tuberculosis in a 28 year old Spaniard. The illness began four years previously with frequent persistent bouts of bronchitis. Two years later a severe stubborn diarrhea appeared and after a few months active pulmonary tuberculosis supervened.

It is, of course, difficult to prove a causal relationship between the pulmonary migration of large numbers of *Strongyloides* larvae and the activation of pulmonary tuberculosis, but the pathology involved in each of the infections makes such a course of events an interesting and significant possibility.

#### TREATMENT

Gentian violet treatment had been successful in eliminating the *Strongyloides* infection, as proved at autopsy. Pulmonary, gastric, duodenal, ileal, jejunal and colonic tissue, as well as tissue from all other organs routinely taken for postmortem examination, were studied microscopically, but no adult worms or larvae were found. At the time of death the patient had received 1.56 grams of gentian violet by mouth over a period of nine days and 0.1 gram intravenously. Larvae had disappeared from the stools by the seventh day of treatment, the day after the medication was given by vein.

The course of treatment suggested by Craig and Fause (1943) consists of 0.06 gram of gentian violet medicinal in one and a half hour enteric-coated tablets t.i.d., one hour before meals until 3.3 grams have been administered.

#### PATHOLOGY

Gross and microscopic lesions attributable to a recently extant *Strongyloides* infection were not found at autopsy although the pathologic picture was somewhat obscured by the tuberculous lesions. But a marked tissue reaction to the presence of *Strongyloides* adults or filariform larvae which are going through a hyperinfecting process is not necessarily to be expected, and the spontaneous destruction of the living worms by a minimal local tissue reaction is apparently a part of the pathology of the infection. Faust et al. (1934) in the pathological study of dogs infected with human

strains of *S. stercoralis* and of rhesus monkeys infected with a chimpanzee strain, describe the encapsulation of female worms individually, the cellular infiltration around the worms and their destruction and phagocytosis. Faust and de Groat (1940) found no cellular reaction other than localized eosinophilia about the adult worms in the intestinal wall of a 12 year old boy who died from an infection in the fourth week of illness.

Paradoxically, the question arises as to the possible connection between the large bowel tumor found at laparotomy nine years before the present patient's death and the *Strongyloides* infection which probably existed at that time. The tumor is described as large and very hard, and it involved the ascending colon and hepatic flexure. Microscopically it was found to consist of organizing connective tissue with many lymphocytes and fewer polymorphs. Examination of many sections later cut from the biopsy specimen failed to reveal the presence of worms or evidence of tuberculous pathology. At autopsy there was no residual evidence to be found of this tumor.

Blacklock and Adler (1922) present findings to show that the presence of *Strongyloides* in the submucosa of the gut may at times cause hypertrophy and tumor formation. At the autopsy of an infected chimpanzee, the jejunum from a point about 12 inches below the pylorus for a distance of five inches was increased to three times its normal thickness throughout its whole circumference; at the commencement of this thickened area there was a three by three by one and a half cm. tumor projecting into the jejunal lumen. Microscopically the tumor was found to be composed of a core of muscle surrounded by a layer of lymphoid tissue extending up to the muscularis mucosae; numerous adult *Strongyloides* were found in the mucosa and lymphoid tissue adjacent to the muscular core.

Price and Dikmans (1929) first reported epithelial tumors of the intestine resulting from *Strongyloides* infection. They found multiple adenomata in the colon of a cat which were caused by an apparently new species of *Strongyloides*. At autopsy numerous small circumscribed nodules were seen beneath the muscularis mucosae. Microscopic study revealed irregular acini of columnar cells supported by delicate connective tissue stroma markedly infiltrated with lymphoid cells. Adult and larval worms were found scattered through the nodules. No adult worms were found apart from the tumors.

## ACKNOWLEDGMENT

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## SUMMARY

1. A fatal case of human strongyloidiasis complicating disseminated tuberculosis is presented, with autopsy findings.

2. The infection was acquired either in Poland or in Rochester, New York; if the former, the infection had maintained itself more than 30 years, through the mechanisms of autoinfection and/or hyperinfection. Further study is necessary to determine the northern limit of the parasite's natural range in this country.

3. The elimination of larval and adult worms from the patient, as proved at autopsy, was effected by 1.59 grams of gentian violet per os and 0.1 gram by vein.

4. The advisability of concentrating liquid stools in parasitological examination is emphasized.

5. *Strongyloides* pneumonitis and the possible rôle played by migrating *Strongyloides* larvae in the activation of inactive pulmonary tuberculosis are discussed.

6. *Strongyloides stercoralis* as an etiologic agent in the production of certain types of large bowel tumors is discussed.

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# THE BEHAVIOR OF TRICHOMONAS VAGINALIS IN A SEMI-SOLID MEDIUM<sup>1</sup>

M. J. HOGG<sup>2</sup>

*Laboratory of Anatomy, University of Pennsylvania, Philadelphia*

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While studying *Trichomonas vaginalis* in tissue cultures (Hogg, 3) we observed a few non-motile forms, each one sending out a long pseudopodium while feeding over the tissue culture cells. This structure always contained the 4 anterior flagella and the undulating membrane. There were a great many free-swimming individuals in other parts of the tissue culture. We suggested that these elongated forms had settled down and were feeding where there was a rich food supply. Pereira and de Almeida (6) found these elongated individuals in several species of trichomonas and called them amoeboid forms. They believed they occur where the organisms are dividing and are in a high state of vitality. They refer to the unpublished work of Pereira and Nobrega, who found these amoeboid forms in lesions of the throats of pigeons and of the oesophagus of chickens, where there was a rich supply of food.

We were carrying our stock cultures of *T. vaginalis* on liver infusion agar slants covered with modified Ringer's solution enriched with human or chicken plasma. Later we began using a semi-solid medium which was a modification of Dr. Garth Johnson's medium<sup>2</sup> but was much thicker than his, as it contained more agar. On this medium a great many of the trichomonads changed into these elongated forms.

There were two distinct parts to these individuals: a spherical or pyramidal part, which was granular, and an elongated, non-granular part, which contained the flagella and undulating membrane (fig. 1, c to d, and figs. 2 and 3). The spherical part was inactive and often had a thick, outer membrane or plasmatheca. It did not change shape, but seemed to be anchored to the coverslip, though occasionally these forms were found free-swimming. The clear, elongated part was free, moving from side to side, sending out small pseu-

dopodia which were quickly withdrawn, or stretching far out into the medium. After it had become extended for several minutes, it would become shorter and be drawn into the body. There would be a quick churning of the cytoplasm in which there was some internal redistribution of materials, and the trichomonas moved off as a free-swimming individual (fig. 1).

In these forms the axostyle, which is attached to the blepharoplast in the anterior end and protrudes from the posterior end, was drawn into the body as the trichomonas elongated. Sometimes a small part of it remained protruding. When it was completely withdrawn the posterior end of the trichomonas often became flat.

Blockmann (1) in 1884 described a double row of granules beginning at the anterior end of *T. vaginalis* and converging toward the posterior end. Since then nearly all workers have seen these granules and have figured them along the axostyle. Wearich (7) considers them to be a species characteristic.

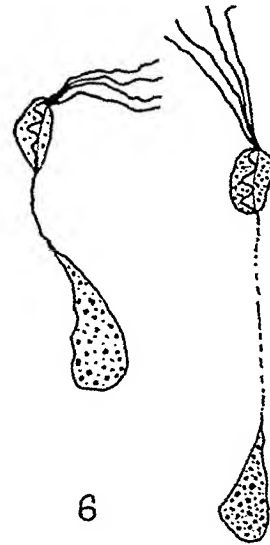
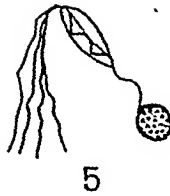
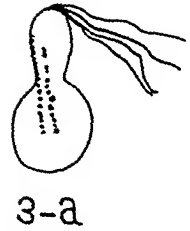
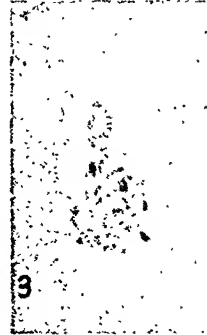
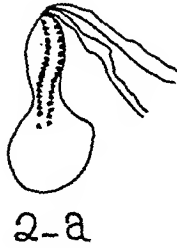
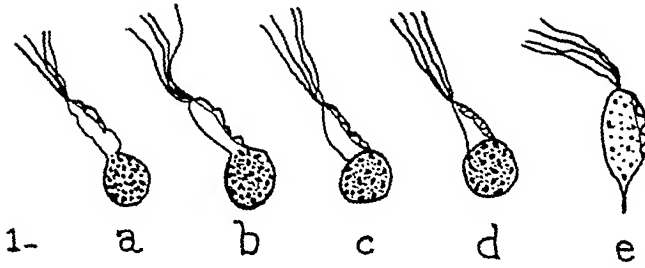
In our elongated forms the axostyle was well defined by one, sometimes by two, rows of granules on either side which looked like strings of pearls and moved as the axostyle moved (figs. 2 and 3). Indeed the shape and position of the axostyle could be determined by these granules. The axostyle was plastic, straight when the trichomonas was elongated or free-swimming, but bent when it was changed from a body to the other.

After a day or two in the semi-solid medium, trichomonads were found with the elongated part stretched out to 10, 15, or 20 times its normal thickness at the end of a culture. These cells had long, broad and flattened flagella (fig. 4). Sometimes a thick thread or pseudopod protruded from the inactive part (fig. 5). Physiological comparisons of these structures were made with the normal trichomonads. The physiological activities of these cells were only the typical trichomonads, swimming, feeding, and dividing. The elongated forms

<sup>1</sup>Read by title at the Thirty-Ninth Annual Meeting of the American Society of Tropical Medicine and Hygiene, Oahu, November 16-18, 1943.

<sup>2</sup>Unpublished, see bibliography.





All drawings were made with a camera lucida.

Fig. 1. A trichomonas showing the spherical granular part with its thick outer layer of plasmagel and the clear elongated pseudopodium. a-d are different stages in the return to a free swimming trichomonas (e). Drawings made about every 3 minutes.  $\times 830$ .

Figs. 2 and 3. Photomicrographs showing the granular spherical part and the clear pseudopodium, also the row of granules on either side of the axostyle. Figs. 2a and 3a are line drawings of the photomicrographs to emphasize the position of the rows of granules.

Fig. 4. A much elongated trichomonas.  $\times 830$ .

Fig. 5. The two parts of the trichomonas are connected with a thin thread of protoplasm.  $\times 830$ .

Fig. 6. Two stages in the degeneration of a much elongated trichomonas.  $\times 830$ .

present but being very thin and inactive, it was hard to see. The four flagella were always visible and were usually extended, though at times they were seen in a knot. Sometimes they appeared shorter, as though contracted. Most of the time there was a slight waving of the flagella, followed at intervals by a quick backward beat. The axostyle did not protrude.

These very much elongated trichomonads were degenerating forms. Many of them were found dead in older cultures. To see whether they could be revived, Ringer's solution was added to the edges of the coverglass preparation. Forms were studied which either had recently shown activity, or whose flagella were still slightly active. As soon as the Ringer's solution was added the flagella began to move, at first gently and slowly and then very strongly and quickly. The undulating membrane began to ripple and gradually the long, neck-like structure became shorter and thicker. The trichomonas stayed in this stage for several minutes. Then very quickly the long pseudopodium was drawn into the spherical part and the trichomonas swam away as a normal individual.

Not all of these much-elongated forms could be brought back to normal. In many cases the degeneration had gone too far (figs. 5 and 6). We found that if the neck-like structure could be revived, then usually the spherical part would become active again. The return to normal activity seemed to begin at the end where the flagella were located; the spherical part, except for an occasional twisting movement, remained inactive until the reorganization of the trichomonas took place.

Death, like the return to normal, was a progressive phenomenon. The tip of the pseudopodium with its undulating membrane and flagella was the last part to move. We have watched this part separate from the posterior end. Both parts soon became very granular and died while the cytoplasmic thread connecting them degenerated into a row of granules which quickly disappeared (fig. 6).

As a check to these experiments a drop of semi-solid agar made up with Ringer's solution was added to a drop of trichomonas in a liquid medium. These were covered with a coverslip and sealed with salvoline. In 24 hours the same kind of elongated forms were present in the thicker medium.

After observing these changes in form we

naturally seek an explanation for this phenomenon and suggest the following one. If we assume that the outer membrane of the trichomonas is a semi-permeable membrane, then in the denser medium the trichomonas loses some of its fluid. As it does this, the outer plasmagel over the body becomes thicker and more granular. Lewis (4) has shown that the contractile tension of protoplasm varies with the viscosity and thickness of the gel layer. So here, as the plasma gel becomes thicker and more viscid it exerts more tension and forces out the long pseudopodium in a place where, as Lewis says, "the elastic strength is lowest." Then as the gel layer on the pseudopodium increases in thickness, it in turn exerts greater contractile tension and the pseudopodium is forced back into the body. At this time the churning of the protoplasm in the body is due to two factors: (a) the movement of the axostyle in the protoplasm as the anterior end of the pseudopodium to which it is attached is withdrawn, and (b) the consequent redistribution of the cytoplasmic granules by this movement.

The immobility of the spherical part is no doubt due to the greater thickness and viscosity of the plasmagel. The trichomonas in this state is comparable to Mast's (5) monopodal amoebae, which were attached only at the tip of the posterior end, so that locomotion was prevented, though there was still a change in shape of the free part. When the trichomonas changed its position it was always by means of its flagella and undulating membrane. The amoeboid motion was limited to the elongated pseudopodium.

Usually *T. vaginalis* has been studied in a liquid medium, where it is free swimming. However, nearly all workers with this form—from Donne (2) on—have noted its ability to change its shape. Some observers have shown pseudopodia being sent out from various parts of the body. These pseudopodia, with the exception of those seen in the microcinematographs of Pereira and de Almeida (6), do not contain the anterior flagella and the undulating membrane and so differ from the ones described in this paper. The long pseudopodium with its flagella and undulating membrane found in the semi-solid medium and in tissue cultures is evidently formed in response to the changed environment.

#### SUMMARY

1. In a semi-solid medium *T. vaginalis* sent out a long, clear pseudopodium containing the 4 anterior flagella and the undulating membrane.

2. This pseudopodium was in constant motion, though the trichomonas did not change its position.

3. The pseudopodium was frequently withdrawn into the body.

4. After 48 hours in the semi-solid medium a much longer pseudopodium was often formed. This could not always be withdrawn and was considered a sign of degeneration. The addition of Ringer's solution sometimes brought this form back to normal.

5. The axostyle with its row of granules on either side was clearly visible in these forms.

6. The density of the medium and the contractile tension of the protoplasm were important factors in the formation of these long pseudopodia.

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# VITAMIN C AND ABILITY TO WORK IN HOT ENVIRONMENTS<sup>1</sup>

AUSTIN HENSCHER, HENRY LONGSTREET TAYLOR, JOSEF BROZEK, OLAF MICKELSEN AND  
ANCEL KEYS

*From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis*

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## INTRODUCTION

The amount of ascorbic acid required for ordinary maintenance of adult man is very imperfectly known. Even the amount needed to prevent scurvy is a matter of dispute (cf. Fox and Dangerfield, 1940). It is agreed, however, that the minimal requirement to prevent clinical scurvy may be far less than needed to allow maximal health and vigor. Recently claims, widely quoted in the popular press, have been made for special requirements for ascorbic acid in high temperatures, particularly in physical work. Further these claims suggest that large ascorbic acid intakes are of immediate benefit in exposures to high temperature of relatively short duration. Considerable pressure has developed to exploit these "benefits" by advertising appeals to individuals and to industries.

This paper is a report on 3 series of experimental studies on 44 normal young men under rigidly controlled conditions of diet, physical work and environment. The ascorbic acid intake was set at two levels: 20 to 40 mg. and 520 to 540 mg. Particular attention was paid to: (a) cardiovascular functions, (b) performance of standard physical tasks, (c) psychomotor functions and (d) ascorbic acid in sweat, blood plasma and urine.

## PROCEDURE

Three main series of experiments were performed. All experiments were carried out during the winter on healthy, "normal" young men who were continuously resident in a controlled tem-

perature suite for the period of the experiments. Standard clothing consisting of shorts, socks and shoes was worn in the work periods.

In Series I a total of 26 U. S. Army soldiers of the 710th M.P. Bn. were studied for one day at a temperature of 78°F., relative humidity 50 per cent of saturation, followed by 3½ days in a hot dry environment in which the temperature was 112°F. in the mornings, 122°F. in the afternoons and 85° to 90°F. at night with the relative humidity kept constant at 25 to 30 per cent of saturation. These men prior to the experiments had been engaged in light garrison duty and had all subsisted on standard U. S. Army Garrison Rations providing 60 to 90 mg. of ascorbic acid per day.

In Series I each half day's work consisted of walking on a motor driven treadmill at 3.25 miles per hour at a 7.5 per cent grade of climb for 6 ten-minute periods alternating with ten-minute rest periods. This work required an oxygen consumption of about 7 times the basal rate. Pulse rates were counted before and for the first 15 seconds after each work period. Crampton "blood ptosis" tests (1920) were made each morning before breakfast and 20 minutes after the end of each morning's work and each afternoon's work. Rectal temperatures were taken after the third and sixth work periods each morning and afternoon. Body weights were recorded each morning before breakfast and after emptying the bladder. The subjects were weighed to  $\pm 7$  grams, nude and dried, before and after the third work period each morning and the sixth work period each afternoon for measurement of the rate of sweating. Twenty-four hour urine output and water intake were recorded for each day. Electrocardiograms and metabolic rates were taken on some of the subjects. Arm-plus-hand sweat was collected in shoulder length "neoprene" gauntlets during the second and third work periods each morning and afternoon. Venous blood samples were taken each morning.

During Series I the subjects received a standard

<sup>1</sup> The work described in this paper was done in part under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Minnesota. Important financial assistance was also provided by the Nutrition Foundation, Inc., by the Corn Industries Research Foundation, by Swift and Company, Chicago, by the National Cane Sugar Refiners' Association and by the National Confectioners' Association. We are grateful to Merck and Company, Inc., for supplies of pure vitamins.

diet of 3000 Calories, normally balanced and adequate, according to current standards, in all vitamins except C which was moderately low—20 to 40 mg. daily. Thirteen of the subjects were given an additional 500 mg. of ascorbic acid daily; the other 13 received placebos. Salt intake was controlled; half of the subjects in both the high and low ascorbic acid groups received 13 to 17 grams of sodium chloride daily while the other men received only 6 to 8 grams.

In Series II 6 college students were studied on 2 occasions 3 weeks apart. In these experiments the men worked on the treadmill for 6 ten-minute periods alternating with equal rest periods. The temperature was 117°F., relative humidity 25 to 30 per cent of saturation. The rate of work was the same as in Series I.

The subjects in Series II ate a normal diet of their own choice except that citrus fruits, tomatoes and other rich sources of vitamin C were excluded for a week before each experimental routine. For 3 days before the first work routine 3 of the subjects were given an additional 500 mg. of ascorbic acid daily while the other 3 subjects received placebos. For the second work routine the order was reversed so that each subject acted as his own control.

The experiments in Series III were precisely like Series I except that the physiological variables were only measured occasionally, attention being centered on psychometric measurements. Twelve subjects were studied in 3 experiments each lasting 4½ days as in Series I. Six of these subjects received a total of about 40 mg. of ascorbic acid daily while the other 6 men received about 540 mg. Again half of each group was on a low salt intake while the other men received a total of 15 to 18 grams of salt daily.

#### METHODS—ASCORBIC ACID

The ascorbic acid content of urine, sweat and blood plasma was estimated by a modification of the method of Mindlin and Butler (1938). Efforts were made to ensure that ascorbic acid destruction in these fluids was avoided.

#### METHODS—PSYCHOMETRIC AND STRENGTH MEASUREMENTS

Psychometric and strength data were obtained in Series III immediately before and after each day's routine on the treadmill. Strength of grip

and of back lift were measured with standard dynamometers.

Flicker fusion frequency (F.F.F.) was measured as a possible indication of central fatigue (cf., Simonson and Enzer, 1941). Manual speed and coordination was tested for 60 seconds with the ball and pipe test (Brozek, 1944). Rate of tapping was measured with an electrical counter which recorded the number of contacts between a stylus and 2 metal plates, separated by 1.7 cm., which were alternately tapped for 30 seconds. Grip strength and F.F.F. were measured 3 times in each test; the other measurements were carried out twice in each test. In order to minimize practice effects training trials were given to each subject on each of the 3 days prior to the start of the experimental period proper. The statistical significance of differences between means of both physiological and psychometric variables was evaluated by the use of the *t*-value (Goulden, 1939).

#### PHYSIOLOGICAL RESULTS

It is not feasible to present the voluminous detailed data. The grand averages for the physiological variables measured in Series I and II are given in tables 1 and 2.

*Pulse rates.* In both Series I and II average work pulse rates were closely similar at all times in the groups on low and on high ascorbic acid intake. Such small differences as appeared in several testing sessions are statistically not significant. In order to economize space standard deviations are given in the tables only for representative sessions but these are typical of all other sessions. The highest individual rates recorded for the first 15 seconds following work were 196 per minute in the low C groups and 204 in the high C groups.

It will be noted (table 1) that with continued stay in the heat the average work pulse rates in both groups were approaching the rates in the (cool) control by noon of the fourth day in the heat. This is the result of acclimatization to the heat and not simple muscular training because the amount of work done was too small to produce a pure training effect of this magnitude within the experimental period. The details of the effects of heat on cardiovascular functions are reported elsewhere (Taylor, Henschel and Keys, 1943). It will also be noted that, in any given half day in the heat, the pulse rate rose progressively from work period to work period (cf. table 2). In

Series II this rise was slightly less in the men on low C than when they were on high C but the difference is of dubious significance.

*Rectal temperatures.* Intense hyperthermia was not produced in any of the subjects in these series. The rectal temperatures averaged 2 to 3°F.

recorded was 103.4°F in a man in the high C group in Series I.

*Crampton tests.* For the present work it was necessary to modify the original Crampton scoring scheme because the vasomotor stability is sometimes very adversely affected in the heat. A

TABLE 1

*Grand averages for the 26 subjects in Series I*

In each experiment Monday was the (cool) control day, the high temperature being started at 8:00 a.m. Tuesday and continuing through Friday noon. "Low C" refers to the men receiving only 20 to 40 mg. of ascorbic acid daily; "High C" refers to the men who received an additional 500 mg. daily. Standard deviations of the means are given for Tuesday afternoons and Friday mornings as representative illustrations.

	MON.	TUES.				WED.		THURS.		FRI.	
	P.M.	A.M.	P.M.	$\sigma =$		A.M.	P.M.	A.M.	P.M.	A.M.	$\sigma =$
Pulse rate:											
High C.....	133	154	163	$\pm 13$		154	159	151	154	140	$\pm 16$
Low C.....	132	158	169	$\pm 18$		157	161	150	154	142	$\pm 17$
Rectal temp., °F.:											
High C.....	100.5	100.7	101.1	$\pm 0.59$		100.8	100.9	100.9	101.2	100.5	$\pm 0.45$
Low C.....	100.4	100.9	101.5	$\pm 0.70$		101.1	101.6	101.2	101.6	100.6	$\pm 0.45$
Crampton Score:											
High C.....	57	63	36	$\pm 17$		55	50	51	50	58	$\pm 20$
Low C.....	59	70	42	$\pm 19$		61	52	52	52	63	$\pm 18$
Rate of sweating, gms./min.:											
High C.....		13.7	13.7	$\pm 2.4$		13.9	12.9	14.5	12.6		
Low C.....		15.4	13.5	$\pm 4.1$		13.2	13.2	13.3	13.8		
24 hr. water drunk (liters):											
High C.....			5.40	$\pm 1.19$			5.95		6.09		
Low C.....			5.02	$\pm 1.16$			5.95		5.88		
24 hr. urine (liters):											
High C.....			0.599	$\pm 0.246$			0.772		0.688		
Low C.....			0.525	$\pm 0.112$			0.571		0.504		
24 hr. sweat* (liters):											
High C.....			6.289								
Low C.....			6.301								

\* Average sweat = (water drunk + water in food + water of metabolism) -- (urine output + water in expired air + water in feces)  $\div$  weight change calculated for the 3 days in the heat and divided by 3 for average daily sweating.

higher in work in the heat than in rest in the (cool) control but the average difference as compared to work in the cool was only 1.2°F. (table 1). There is a slight advantage on the second day in the high C group in Series I but this is reversed in Series II. The highest individual temperature

"high" score indicates better adjustment of the vasomotor system. In the cool the average of normal young men is a score of 55 to 60. In general the men on high C tended to have poorer Crampton scores than the men on low C and this was apparent at all times in both Series I and Se-

ries II. However this difference is of doubtful significance in view of the large individual range. For example, in Series I individual Crampton scores ranged from 10 to 100 for the low C group and from 5 to 95 for the high C group.

*Rate of sweating.* Individual rates of sweating in Series I varied from 7.7 to 32.1 grams per minute or 462 to 1926 cc. per hour with a general average of about 800 cc. per hour. Both the rate of sweating in work and the daily (24 hour) sweat volume were substantially identical in the low C and the high C groups. It is interesting that there was little or no progressive change in rate of sweating in either group during the period when substantial acclimatization to the heat was being achieved.

high C group. At the same time the low C group excreted only from 4.3 to 9.9 mg., with an average of 7.6 mg. The plasma ascorbic acid in the high C group varied from 0.43 to 1.48 mg. per 100 cc., with an average of 1.21 mg. At the same time the low C group ranged from 0.22 to 1.23 mg. of ascorbic acid per 100 cc. of plasma with an average of 0.65 mg.

*Water intake and balance.* Daily water intakes ranged from 3.05 to 8.55 liters. The average water intakes for the low and the high C groups however did not differ by more than 400 cc. on any one day in the heat. The high vitamin C group drank an average of 143 cc. more water per day and excreted an average of 153 cc. more urine. Neither group drank sufficient water to

TABLE 2  
Grand averages for Series II

Six subjects who were their own controls on high and low vitamin C intakes worked in the heat at 120°F. for 3 hours, with three weeks between the exposures.

WORK PERIOD. (10 min.).....	1	$\sigma =$	2	3	4	5	6	$\sigma =$
Work pulse rate, beats per minute								
On high C.....	154	$\pm 12$	161	160	166	170	174	$\pm 17$
On Low C.....	159	$\pm 15$	161	163	163	168	171	$\pm 16$
Rectal temperatures, °F.								
On High C.....	100.0	$\pm 0.52$	100.3	100.9	101.3	101.4	101.6	$\pm 0.94$
On Low C.....	99.9	$\pm 0.93$	100.5	100.8	100.8	101.1	101.1	$\pm 0.95$
	Morning				Noon			
Crampton score.....								
On high C.....	57.5 $\pm 5.6$				44.2 $\pm 3.1$			
On Low C.....	61.6 $\pm 4.4$				50.8 $\pm 6.4$			

*Ascorbic acid in sweat, urine and plasma.* The concentration of ascorbic acid in the sweat was generally less than 0.1 mg. per 100 cc., the average being 0.059,  $\sigma = \pm 0.047$  in the men receiving 520-540 mg. daily, and 0.060,  $\sigma = \pm 0.056$  in the men on low C intake. Somewhat lower values were obtained when the greatest efforts were made to prevent vitamin C loss and to exclude non-ascorbic acid reducing substances. The details of these studies are reported elsewhere (Mickelsen and Keys, 1943).

Urine and blood plasma ascorbic acid concentrations reflected the vitamin C intakes as expected. On the last day in the heat the excretion of ascorbic acid in the 24-hour urine ranged from 161 to 513 mg., with an average of 222 mg. in the

maintain complete water balance, but the average daily weight loss was almost exactly identical in the 2 groups—465 and -467 gms. in the high and low C groups respectively. This loss in body weight was accompanied by a slight increase in hemoglobin concentration amounting to an average of 0.2 gm. per 100 cc. of blood in both groups.

#### RESULTS—STRENGTH TESTS

The grip strength and back lift tests in Series III showed no important difference between the men on low C as contrasted with those on high C, which might be referred to the level of vitamin C intake. It happened that the men on the low ascorbic acid intake were initially slightly stronger, or more capable of exerting their strength, than those in

in the high C group, and there was a tendency for this advantage to be reduced in successive days in the heat. However, this apparent difference in response was small and not statistically significant. For example, the average back lifts were initially 271 and 290 lbs. in the high and low vitamin C groups, respectively; after 3 days in the heat and the averages were 286 and 292.

#### RESULTS—PSYCHOMETRIC MEASUREMENTS

No statistically significant difference between the high C and the low C groups was found for any period in the tests of rate of tapping either in the first 10 seconds or in the last 10 seconds, nor were there any progressive trends. The same was true of the manual speed (pipe) tests, though in the latter case both groups tended to show a slight training improvement.

less (1938) demonstrated a more rapid depletion of body stores of ascorbic acid in heated guinea pigs than the controls where both groups of animals were maintained on a scorbutic diet. The same authors showed that urinary excretion of the vitamin is diminished in humans by treatment in a fever cabinet. Daun, Boyd and Paul (1939) reported a decrease in both urinary and plasma ascorbic acid in man in a fever cabinet. Falke (1939) claimed that artificial fever increases the vitamin C requirement by 100 mg. per day. However, Osborne and Farmer (1942) could find no reduction in plasma C in patients undergoing fever therapy and emphasized the questionable nature of urinary excretion as an indicator of bodily requirements.

Elsewhere (Mickelsen and Keys, 1943) we have reported that sweat contains at most only insignificant

TABLE 3

*Flicker fusion frequency values, in flickers per second, in Series III for the men receiving a total of 20–40 mg. ascorbic acid daily (Low C), and those receiving 520–540 mg. (High C)*

The t-value for the 5% level of significance is 2.23

STATISTICAL VALUE	DAYS IN THE HEAT					
	I		II		III	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Mean, High C.....	48.7	48.8	49.1	50.3	49.8	50.3
Mean, Low C.....	49.3	49.6	49.3	48.8	47.6	48.0
Absolute difference.....	+0.6	+0.8	+0.2	-0.5	-2.2	-2.3
Stand. dev. of diff.....	±1.48	±1.55	±1.53	±1.68	±1.42	±1.52
t-value.....	0.405	0.516	0.130	0.298	1.55	1.51

The flicker fusion (F.F.F.) test results are given in table 3, together with the results of statistical analysis. There was a slight but consistent tendency for F.F.F. to improve in the men on the high C intake during successive days in the heat and an equally slight but consistent tendency for the men on the low vitamin C intake to deteriorate.

#### DISCUSSION

The idea of an increased need for ascorbic acid in heat probably originated from observations on blood and urinary levels of the vitamin in hyperpyrexia and on the concentration of the vitamin in the sweat. Abbasy, Hill and Harris (1936), reporting decreased blood and urinary ascorbic acid in hyperpyrexia of infective origin, suggested that an increased demand for or destruction of vitamin C exists under this condition. Zook and Sharp-

nificant amounts of ascorbic acid. As a matter of fact, with the exception of Cornbleet, Klein and Pace (1936) and Bernstein (1937), other investigators have reported only small amounts of ascorbic acid in sweat (Zselyonka, and Manassy-Mégay, 1937; Wright and MacLenathen, 1939; Hardt and Still, 1941) or none at all (Tennent and Silber, 1943).

The direct claim that a high intake of ascorbic acid reduces heat disability has been given much attention (cf. Anonymous, 1942; Holmes, 1942) but it appears to be supported chiefly by trials made by one industrial organization. Field studies on negro laborers in hot climates by Fox (1942) failed to indicate any special need for an elevated intake of vitamin C but detailed observations were not made.

In the industrial trials referred to above the



workers were exposed to various concentrations of toxic chemicals as well as to heat. It is possible that the high vitamin C intakes may have a beneficial effect on the cardiovascular system (as expressed by the Crampton test) under those conditions which would not strictly be an effect on the cardiovascular adjustments to high environmental temperatures. In fact, an analysis of the data on the preliminary trials that have been made available to us through the kindness of Dr. J. H. Foulger of the Haskell Laboratories of Industrial Toxicology, Wilmington, Delaware, are suggestive of such a possibility.

Heat exhaustion characterized by nausea, vomiting, dizziness, weakness, a rapid feeble pulse, and inability to continue work occurred in 6 of the 13 subjects on low chloride intake in Series I. Three of the cases were in men in the high C intake and three were in the low C intake. The high C intake did not protect against heat exhaustion. Five of the six heat collapse cases in our studies recovered sufficiently within 24 hours to continue work without any treatment except rest even though exposed to the high temperatures during recovery.

The present studies clearly show that there is no significant beneficial effect of high ascorbic acid intake on ability to work in the heat within the duration of our experiments. Our results do not prove that a high ascorbic acid intake may not be beneficial in a very prolonged stay in the heat. However, there is no acceptable evidence for such a long-time effect.

#### SUMMARY

1. The performance of muscular work in dry heat—up to 122°F.—was studied in 44 normal young men under rigidly controlled environmental, dietary and work conditions. The stay in the heat varied from 3 hours to 4 days.

2. Comparisons were made between performances on a diet restricted in ascorbic acid intake and a diet supplemented by 500 mg. ascorbic acid daily. The dietary differences were maintained for periods of 4 to 7 days.

3. Pulse rates in rest and in work, rectal temperatures, vasomotor stability tests, rates of sweating, general observations and subjective reports all failed to demonstrate any significant advantage for the men receiving supplements of ascorbic acid.

4. Psychomotor tests and strength tests likewise generally failed to show any advantage in the ascorbic acid supplementation. There apparently was a slight gain in flicker fusion frequency related to the extra intake of vitamin C.

5. Daily sweat losses were of the order of 5 to 8 liters but the total loss of vitamin C in the sweat is entirely negligible.

6. Heat exhaustion occurred with equal frequency in the vitamin C restricted and supplemented groups.

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# MEDICAL CARE IN THE BELGIAN CONGO<sup>1</sup>

CHARLES A. FLOOD<sup>2</sup> AND WILLIAM SHERMAN<sup>3</sup>

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In a period of a little more than fifty years, an excellent medical service for the care of the African native has been developed in the Belgian Congo. This service is of particular interest because of the manner in which it has dealt with the unusual problems of medical care of a semi-civilized people in the heart of the tropics. It is a government operated service with primary emphasis on public health work and is staffed in part with trained native medical personnel in all departments.

There is, in the Congo, a very high incidence of infectious and parasitic diseases, both the typically tropical diseases and those found in the temperate zones as well. These diseases are so common and the mortality from them is so great that degenerative disorders are comparatively insignificant in number. No figures on life expectancy of the natives can be gathered, as most of them do not know their own ages, but it is certainly much less than that of white races. A large proportion of the illnesses fall in the category of intestinal diseases, the spread of which is favored by poor sanitary conditions and the insect-borne diseases, the vectors of which are very numerous. The eradication of these communicable diseases has been the foremost aim of the medical service and more emphasis has been placed on preventive medicine than on the care of the sick individual.

Inasmuch as the natives are able to pay little or nothing for medical care, it is essential for the government to supply medical service for them. A system of government administered medical care has been developed, supplemented by the medical services of the large commercial organizations and by the foreign missions which also operate hospitals and dispensaries. There are a few private practitioners in the Congo but the number of white people, even in the larger cities, is not great

enough to attract private physicians from Europe. Medicine as it exists in the Congo today is a modified form of state medicine, administered and paid for by the government.

The number of doctors and white nurses in the colony has always been very small in relation to the size of the population. When one considers that the natives live for the most part in small villages scattered sparsely over a wild country it is apparent that the difficulties of making medical care available throughout the colony are very great. In order to increase the number of medical personnel, the government and also the foreign missions have undertaken to train natives as medical assistants and nurses. These native auxiliaries become very proficient in such duties as bedside nursing, obstetrical deliveries, routine laboratory procedures and the administration of parenteral medications under the direction of European doctors and nurses. The native medical personnel are educated in schools known as the "medical schools of inferior degree."

## ORGANIZATION OF MEDICAL SERVICES

The government medical service is similar in organization to that of an army. Authority is vested in the medical director of the colony, the *medecin en chef*. The latter is in turn responsible to the governor-general of the colony, to whom he suggests changes in personnel, submits the provincial budgets for the cost of medical care and reports on the state of sanitation in the colony. During peace times, the *medecin en chef* is also in charge of the medical department of the colonial army. The service has three branches, the practice of medicine, the public health service and the research department and its physicians are assigned to one of these three departments. Each of the six provinces of the colony has a medical director responsible to the *medecin en chef*. With this system of centralized control an optimum distribution of available physicians throughout the colony is possible. Country-wide surveys of disease distribution and various programs for the control and treatment of communicable diseases can be effectively coordinated.

<sup>1</sup>We are indebted to Colonel Lucien Van Hoof, *Medecin en Chef du Congo Belge*, and members of his staff for the statistical data which is quoted in this paper and for the opportunity of observing a number of hospitals, clinics and laboratories in the colony.

<sup>2</sup>Major, Medical Corps, United States Army.

<sup>3</sup>Major, Medical Corps, United States Army.

In the year 1940, there were 201 physicians working for the state. In addition, there were 188 public health officers, the "gardes sanitaires" many of whom in addition to their other functions carry out routine physical examinations and prescribe treatments for the natives under the direction of physicians. The medical staff is extremely small to supply the needs of a population estimated at 10 millions.

The government hospitals are staffed by physicians, nurses with administrative or nursing training and native medical auxiliaries. For example, the largest hospital in Leopoldville, the capital of the Belgian Congo is an institution with approximately 250 beds. On the medical service of over 100 beds, there is one physician who because of his additional duties is able to devote only a few hours of the day to the hospital. Much responsibility therefore falls upon the nuns who are in charge of the wards. The nuns are experienced in eliciting histories, recognizing gross physical signs and become familiar with the clinical pictures of most of the prevalent diseases. When necessary they prescribe standard treatments pending the daily rounds of the physician.

Similarly in the dispensaries, the burden of the work is carried by the nuns with the aid of native nurses. One of the clinics in Leopoldville, as an example, takes care of an average of 200 patients each morning. The patients are all seen by a sister who elicits the history, briefly examines the patient and prescribes standard treatments which are usually administered by one of the five native nurses who assist her. Among the commoner diseases seen in such a dispensary are malaria, particularly in babies, minor surgical conditions, tropical ulcers of the leg, diarrheal diseases and infestations with parasites such as hookworm and ascaris.

The foreign missions also do a considerable share of the medical work in the Congo. Distributed throughout the colony are a large number of hospitals and dispensaries belonging to the various Protestant denominations. Their method of operation is essentially similar to that of the government installations. Native assistants and native nurses are extensively employed to assist the missionary doctors and nurses.

Most of the large industrial, transportation and trading companies supply medical care to their own employees, both white and native. Doctors are employed on a contract basis and full medical

care is furnished for all illnesses of employees and their dependents. The physicians make home calls on the white employees when necessary and give them the same service they would expect from a private practitioner. The doctors employed by the larger companies are among the outstanding medical men of the community, so there is little inclination even among the highest paid European employees to seek private medical care. Contract physicians and also the government doctors are permitted nevertheless to care for private patients and to receive remuneration for their services. In 1940, there were 81 doctors in the Congo working for private companies.

#### MEDICAL EDUCATION OF NATIVES

The medical schools of inferior degree which are operated by the government for the education of native medical assistants and male nurses are in effect nursing schools. The curriculum includes all of the subjects which are taught in European medical schools although in less detail, and the teaching is done by doctors, public health officers and nurses who are assigned to the various medical installations in the surrounding area. Instruction is largely didactic and consists of lectures, demonstrations and laboratory exercises in premedical subjects, the basic medical sciences and the clinical branches of medicine. The didactic courses are given over a three year period for the medical assistants and a two year period for the nurses. This is followed by two years of practical experience in hospitals, dispensaries and laboratories. At the end of this time a difficult final examination must be passed. The students acquire a general knowledge of medicine as well as proficiency in nursing procedures, laboratory diagnosis and minor surgical techniques and are prepared to work as nurses, technicians or medical assistants under supervision. The medical assistants are permitted to assume more responsibility than the nurses because of their wider training.

The cost of medical education is borne entirely by the government which also pays for the living expenses of the students. Students are subject to rigid military discipline and infractions of rules are punished by fine or expulsion.

The foreign missions also operate medical schools for the training of native auxiliaries. The course of instruction is similar in all respects to that offered by the government schools.

There are several other types of native medical

auxiliary who receive their instruction in the various hospitals, dispensaries and laboratories. These include the obstetrical aides who are trained in midwifery, the nurses aides and the native "gardes sanitaires" who assist the European public health officers. The period of instruction for these auxiliaries is shorter than that for the nurses and emphasis is placed primarily on practical work in the particular field of specialization.

#### PUBLIC HEALTH WORK IN THE CONGO

Malaria is probably responsible for more morbidity than any other disease in the Belgian Congo. Careful studies have shown infection with malarial parasites to be almost universal among the natives. One such study carried out in an area east of Leopoldville showed a 98 per cent plasmodial index among native babies with a gametocyte index of 8 to 15 per cent. The splenic index in the children in this region was 47 per cent. Forty-five per cent of the natives of all ages were found to have parasites in the blood at the time of examination. Observations such as these indicate that practically all individuals have had malaria and are subject to reinfections. Similar conditions prevail throughout the Congo except in the areas of high altitude in the eastern portion.

There are no reliable figures on the incidence of clinical malaria in the natives but all cases of fever without other apparent cause are considered malaria and treated with quinine usually without examination of the blood being undertaken. Of 22 Europeans dying in the Belgian Congo in 1940 of tropical diseases, 20 succumbed to malaria and its complications.

Throughout the lower part of the Congo, mosquitoes of many species are exceedingly common. Several of these act as vectors of malaria but the most important is *Anopheles gambiae*. Because of the type of terrain with many rivers and swamps, eradication throughout the colony as a whole is impossible. In the larger European towns, careful surveys of the prevalence of the various types of mosquito and of the incidence of malarial infection in the susceptible species are carried out constantly, and much has been accomplished by eradication of breeding places, oiling of water and other control measures. The segregation of the natives in villages removed from the European residential sections also helps to lessen the exposure of the white population to infected mosquitoes.

Even in such a town as Leopoldville, however, chemical suppressive treatment is required to control the incidence of clinical malaria and in the remote portions of the colony where mosquito control is not feasible, the control of the disease must depend for the most part on suppressive therapy. Quinization is recommended for all children up to the age of 5 or 6 years. After that age, the importance of administering quinine depends on the incidence of the disease in a given area. The difficulties of such a program, the amount of quinine, the personnel needed and the problem of educating the natives are apparent. The need for a supply of quinine is apparently being met by the development of cinchona plantations which are reported to be sufficient to supply the current needs in the Congo. Studies are being carried out on the use of totaquina, a cruder preparation of cinchona alkaloids which is more easily prepared from the bark than pure quinine.

Yellow fever is well controlled in the Congo, although the insect vector, *Aedes aegypti* is common. No cases of this disease were reported between 1937 to 1940 and only occasional cases since. All suspected cases that die are examined by autopsy and the liver is studied for evidence of the disease, but most of them are shown to have malaria with jaundice. Surveys of the population with serum protection tests nevertheless show evidence of antibodies in many individuals, suggesting that they have had latent or subclinical infections. The conflict of such tests with clinical observations has not been adequately explained.

Yellow fever exists in adjacent French Equatorial Africa and the Anglo-Egyptian Sudan, and within the past 30 years there have been outbreaks in the Belgian Congo. Definite laws have been passed detailing the management of communities where any epidemic contagious disease threatens. The district commissioner, acting on medical advice is required to take all necessary steps to prevent the spread of the disease. All individuals who are sick from any cause must report the fact to the authorities or to their physicians and no one is permitted to leave the area during an epidemic. During yellow fever epidemics, suspected cases and contacts are isolated, travellers in the area are detained for six days, loitering in public places after dark is prohibited and the huts belonging to patients with yellow fever are burned down.

Despite the successful control of yellow fever,

an extensive program of vaccination against the disease is being carried out, especially among the European residents and the members of the colonial army.

An important part of the public health program is that devoted to the control of African sleeping sickness. Since this is an insect-borne disease, an attempt is made to eradicate the breeding places of the vector, the tsetse fly, in the populated areas. This is accomplished by clearing brush from the banks of rivers and lakes in the vicinity of villages and plantations. Such measures have proven effective in the vicinity of the larger cities, but the size and nature of the country makes it impossible to achieve this throughout the colony. In some cases, native villages in highly infested areas have been moved completely to more favorable locations, and regulations have been set down against fishing in waters where tsetse flies are known to be abundant.

The main reliance in the program of control, however, is on early diagnosis of human cases and treatment with Tryparsamide or Bayer 205, which renders them non-infective. The onset of the disease is so insidious, that the only practical way of detecting the early cases is by routine physical examinations. Therefore, the government health service attempts to examine all natives once a year with the assistance of the missionary societies and the Foréami (Fonds Reine Elizabeth pour l'assistance medicale aux indigenes du Congo Belge), an organization which devotes the major portion of its endowment to the control of sleeping sickness. As a further check to prevent the spread of the disease from highly endemic to relatively free areas, all natives who travel from one district to another are required to obtain a medical passport certifying that they are free of infectious diseases including trypanosomiasis. While it is impossible in such a large and undeveloped country to reach all the natives, the number seen on the surveys is surprisingly large and the results show a satisfactory decrease in the incidence of trypanosomiasis from year to year. In 1927, 1,700,000 natives were examined and more than five per cent found to be infected. In 1940, almost 5,000,000 were examined and less than one per cent were infected. However, if the control program is neglected in any one area for several years, the disease rate rapidly rises to former levels.

Yaws constitutes a major public health problem, numerically much more important than syphilis.

Yaws occurs chiefly in the rural districts and is extremely common in some areas. The Fonds social de Divu, a privately endowed organization, devotes its resources to the control of the disease in the eastern part of the colony. All persons with skin lesions suggestive of yaws are required by law to report for treatment. During 1930, there were 316,000 cases known to be under treatment; by 1940 the number had fallen to 226,000.

Strict laws are set down for the control of venereal disease among the Congo natives, and a number of special clinics for the treatment of these diseases are conducted by the Red Cross. There is compulsory registration of all "filles publiques," their activities are regulated by law, and they are required to carry a sanitary record showing the results of periodic medical inspections. Furthermore, all natives entering the cities from rural districts and all immigrants arriving from the adjacent French and Portuguese colonies are required to have blood Wasserman tests. Blood tests are also done on all women treated in prenatal clinics. It is recognized that positive reactions may be due to preexisting yaws as well as syphilis, but in all doubtful cases treatment for syphilis is instituted. When the diagnosis of syphilis has been made, treatment is compulsory, and failure to report to the clinic results in arrest by the police and suitable punishment. The treatment is carried out chiefly with neoarsphenamine and bismuth. Statistically it appears that syphilis is being held in check. In 1930, there were 10,697 cases reported, with 100 deaths attributed to the disease; in 1940, the number of reported cases was 12,169 with 142 deaths. The reported incidence of gonorrhea in 1930 was 16,240 cases; by 1940, the number had increased to 26,781. The habits of the African native permit such freedom of extramarital intercourse that the control of venereal disease is a difficult problem.

Another common and serious disease in the Congo is leprosy. There were in 1940, over 68,000 cases of leprosy under treatment. The lepers are either kept in confinement in leprosaria or under close supervision while the children of lepers are removed from their parents and cared for by the state. The Red Cross has also established numerous leprosaria and grows its own chaulmoogra. The cases of leprosy are commonly discovered in the course of the surveys of the population for sleeping sickness.

Tuberculosis has not been spreading in the

Congo as rapidly as it has in some other parts of Africa. In order to prevent the introduction of tuberculosis into the colony, a law was passed in 1921 requiring every newcomer to present a medical certificate stating that he was free from this disease. Inhabitants of the colony who have inactive tuberculosis are required to undergo a medical examination every six months. Since the outbreak of the present war, it has not been possible to send Belgians who develop tuberculosis back to the mother country, as has been the former practice, and the hazard of spreading the disease has been increased. The natives who report to the dispensaries with symptoms of tuberculosis are usually found to be in a far advanced stage of the disease. Reluctance on the part of the natives to seek medical care for fear that they will be removed from their tribes and hospitalized, together with the difficulties of early diagnosis without extensive use of X-rays are factors which account for an increase in the incidence of the disease. In 1940, there were 877 new cases reported with 452 deaths. Ten years earlier there had been 652 new cases with 261 deaths from tuberculosis.

Small-pox is a serious problem, despite efforts to vaccinate as many of the natives as possible. Since 1894, vaccination of all colored workers has been required by law. In 1940, for example, 847,647 natives received initial small-pox vaccinations, while 738,118 were revaccinated. A number of difficulties are encountered in the vaccination of natives. The small-pox vaccine is difficult to preserve in the heat of the tropics. The natives often attempt to neutralize the vaccine by exposure to the sun, or by the application of pieces of citrus fruits, or by mechanical means. Furthermore, the Jenner vaccine apparently does not pro-

duce satisfactory protection against alastrim, which appears to be a modified milder form of small-pox. Despite the vaccination program, there are usually 40 to 50 cases of small-pox in the hospital for contagious diseases at Leopoldville, and the disease sometimes assumes an epidemic form in outlying districts where there are more unvaccinated natives.

#### SUMMARY

Medical care in the Belgian Congo is provided chiefly by the government, supplemented by the work of the foreign missions and the medical departments of large industrial concerns. The government service resembles a military organization in structure and mode of operation. Native medical auxiliaries are trained by the government and by the missions and are employed to assist the white doctors and nurses.

The medical resources have been devoted primarily to the prevention of communicable diseases. A program of yearly mass surveys of the population has been evolved for the purpose of diagnosing and treating the carriers of certain diseases, notably trypanosomiasis. The population is being immunized against yellow fever. Eradication of insect vectors of disease is accomplished in limited areas of the colony where this is feasible.

Apart from humanitarian considerations, the conservation of native manpower is essential for the future development of the country. The medical program is part of the larger government policy of protecting, gradually educating and civilizing the natives of the Congo under a regime which has made this one of the model colonies in Africa.





## AN APPARATUS TO FACILITATE THE FEEDING OF INSECTS ON LABORATORY ANIMALS<sup>1</sup>

ARDZROONY PACKCHANIAN

*From the Department of Preventive Medicine and Public Health, The School of Medicine, University of Texas, Galveston, Texas*

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The transmission of infectious diseases by insects is a vitally important study in epidemiology and preventive medicine. The scientist often needs to raise and propagate various insects in large numbers and to study their ability to transmit certain infectious diseases. For these purposes the writer has constructed an apparatus to facilitate the feeding of insects on animals such as rabbits and monkeys.

### CONSTRUCTION OF THE INSECT-FEEDING APPARATUS

This device consists of three parts as illustrated in figure 1. Parts A and B are identical in construction and are fastened to opposite sides of the animal board (fig. 1, D) by means of brass rods and screw cups (fig. 1, b, c, and d). Part C is rectangular in form, and has several vertical openings to accommodate test tubes or tumblers varying from 25 to 80 mm. in diameter (fig. 1, c).

When insects are to be fed vertically on an animal, the ends of Part C are firmly connected to the tops of parts A and B with the aid of hinges (fig. 1, a). On the other hand, when it is desired to feed insects laterally on either or both sides of an animal, the ends of part C are inserted into the oblique grooves of parts A and B (fig. 1, f). The feeding unit (A, B, and C) can be raised and lowered as desired (fig. 1, b, c, and d).

### METHOD OF HOLDING EXPERIMENTAL ANIMAL ON BOARD

The feeding unit can be used conveniently in connection with an animal board constructed by the writer (1). This board is adjustable for large

and small laboratory animals, is easy to operate, and facilitates the feeding of insects without the constant attendance of an operator. The rabbit or monkey is securely fastened on this board with the devices provided, and experiments of several hours' duration can be conducted without hurt or discomfort to the animal. (See figs. 2, 3, and 4.)

### THE FEEDING OF INSECTS ON EXPERIMENTAL ANIMALS

After the animal is placed on the table, the hair is clipped. Insects which can feed through gauze are placed in a test tube or tumbler. A fine quality of gauze (or wire mesh) is fastened over the container with rubber bands and tape to prevent the escape of the insects or their eggs. The inverted tumbler or test tube containing the insects is inserted through the appropriate opening of part C onto the hair-clipped skin of the animal, thus permitting the insects to feed through the gauze. (See figs. 2, 3, and 4).

### SUMMARY

This new insect-feeding apparatus is especially helpful in maintaining an adequate supply of blood-sucking insects for scientific studies. It is used successfully by the writer in feeding several species of reduviid bugs (*Triatoma*) and yellow fever mosquitoes (*Aedes aegypti*) on experimental animals. This apparatus is simple in construction, inexpensive, and useful in studying certain entomological problems and the transmission of various infectious diseases by insects.

### REFERENCES

- <sup>1</sup>A part of this work was done during 1936-1941 at the National Institute of Health, U. S. Public Health Service, Washington, D. C.
- (1) PACKCHANIAN, A.: Animal Board for Guinea Pigs, Monkeys, Rabbits and Other Laboratory Animals (1942). (In press.)

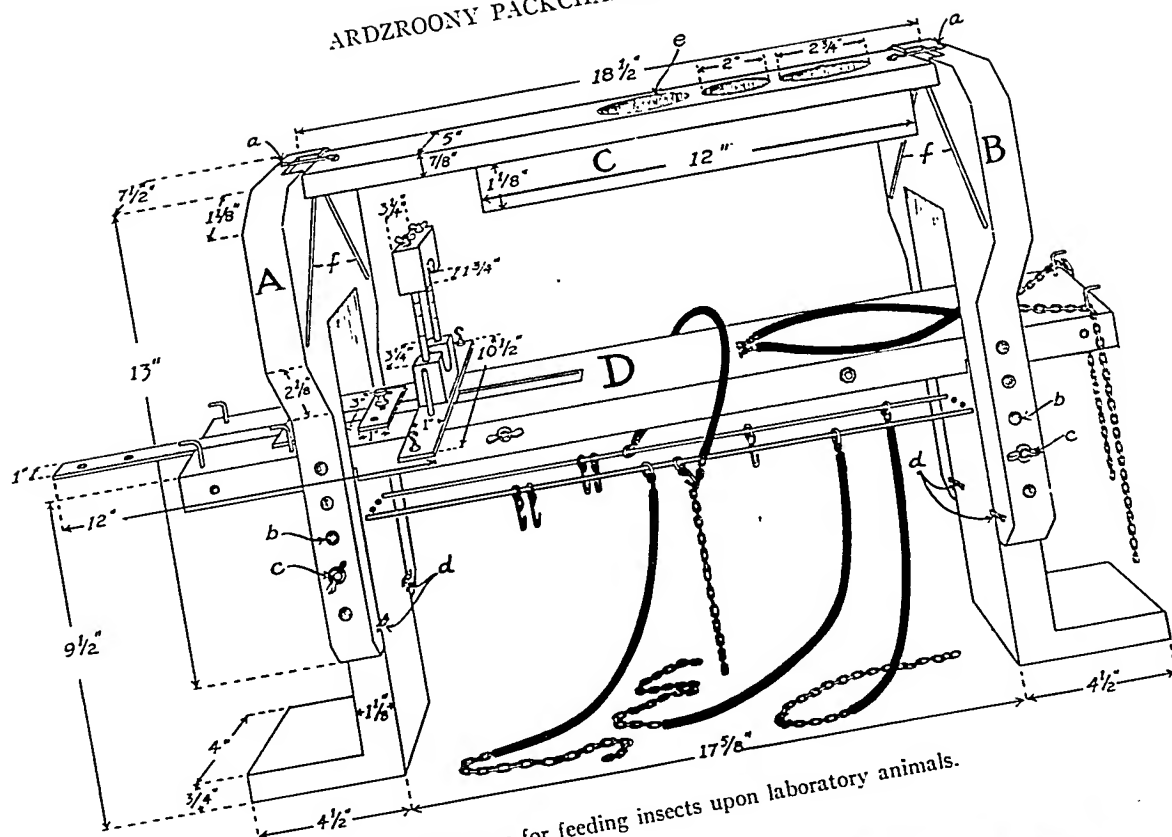


FIG. 1. Apparatus for feeding insects upon laboratory animals.

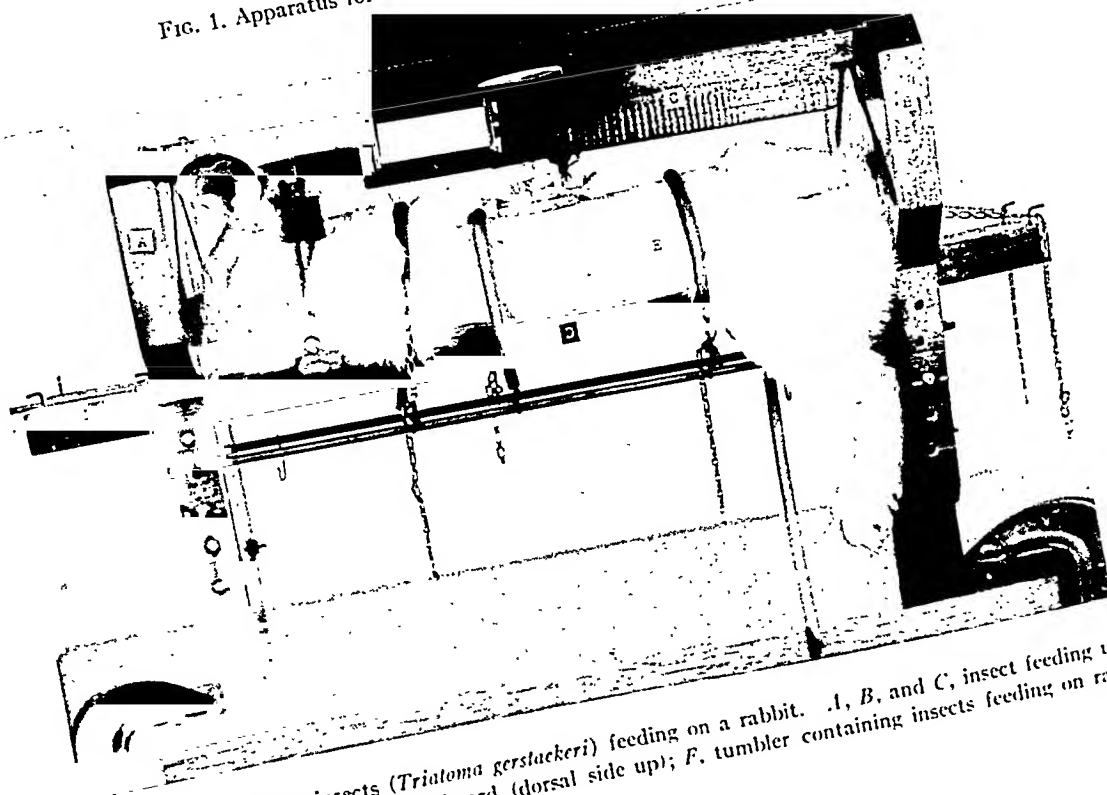


FIG. 2. Blood sucking insects (*Triatoma gerstaeckeri*) feeding on a rabbit. A, B, and C, insect feeding unit; D, animal board; E, rabbit held on board (dorsal side up); F, tumbler containing insects feeding on rabbit through gauze.



FIG. 3. Blood sucking insects feeding on a rabbit. A, B, and C, insect feeding unit; E, rabbit held on animal board (ventral side up); F, tumbler containing insects feeding on rabbit through gauze; G, tumbler containing insects after meal of blood.

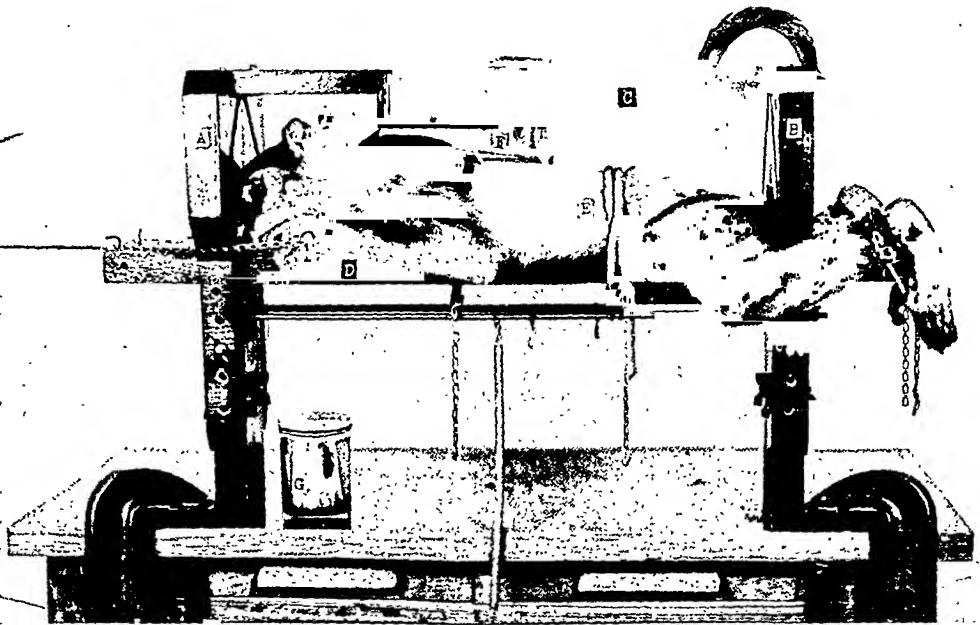


FIG. 4. Blood sucking insects feeding on monkey. A, B and C, insect feeding unit; E, monkey held on board (dorsal side up); F, tumbler containing insects (*Triatoma*) feeding on monkey through gauze; G, *Triatoma* in tumbler after meal of blood.



## BOOK REVIEWS

*Clinical Tropical Medicine.* By twenty-seven authors, edited by I. TAYLOR BEROVITZ, M. D., foreword by WILLIAM A. SAWYER, M. D., Pp i-xvii, 1-957 with 188 illustrations, 20 plates 6 in full color. Advisory Editorial Board—Col. Charles F Craig, M. D., U. S. Army (Retired), W. W. Cort, A.M., PhD. Col. Edward B. Vedder, M. D., U. S. Army (Retired). Paul B. Hoeber. New York and London, 1944.

This book on "Clinical Tropical Medicine" was designed as an aid to the general practitioner in diagnosis and treatment; as a handbook of prevention for the medical officers in the Armed Forces, and as an introduction to tropical disease symptomatology, pathology and treatment for the medical student. It is difficult for any one book to fulfill adequately all of these aims. In general this text tends to emphasize the discussion of the etiological agents and diagnosis. The discussions of symptomatology, treatment and prevention are in certain chapters very brief.

In any book written by numerous authorities, there is great variation in the style and presentation of the various subjects. Certain outstanding authorities maintain the excellence and completeness of their presentations. The presentations of Relapsing Fever, Infectious Jaundice, Rat Bite Fever, Seven Day Fever, Dengue and Phlebotomus Fever by the well-known writer Col. C. F. Craig are recognizable in these respects. The presentation of Rickettsial Diseases by Henry Pinkerton and of Yellow Fever by Fred L. Saper are excellent.

The discussion of snakes, snake poisoning and management of snake bite by James A. Oliver and Dudley Jackson is well-organized and presents a clear discussion of the most accepted treatment.

There are 46 pages and 13 full page plates devoted to laboratory methods and description of the intestinal protozoa. Col. George Callender's articles are extensively quoted in the pathology of the dysenteries.

There are 46 pages with 4 full page color plates on malaria. In the chapter on malaria, quinine dihydrochloride is recommended for intravenous use in doses of 165 gm. (25 grs). This is dangerously in excess of the dosage recommended by most authorities. The prophylactic dose of 0.1 gm. atabrine three times weekly would probably be inadequate in the majority of cases.

The nutritional diseases are well presented by Colonel Edward B. Vedder. This section is well-organized and concise.

There are 103 pages devoted to diseases caused by yeast and fungi. The superficial mycoses, dermatomycoses and systemic mycotic diseases are well presented by Morris Moore. A discussion of these important diseases is desirable but the space given to them in this text seems disproportionally large.

There are only 91 pages devoted to all the helminthic diseases. In view of the increasing importance of some of these infestations, i.e., filariasis, the discussions of these subjects are very brief. The chapter devoted to treatment of intestinal worms discusses complete treatment regimes for the anthelmintic drugs.

There are short discussions of Personal Hygiene and Sanitation in the Tropics.

FRATIS L. DUFF.

*Tropical Nursing.* A Hand-Book for Nurses and Others going Abroad. By A. L. GREGG. M.A., M.D., M.Ch., B.A.O (Dublin). D.T.M. and H. (Lond.), L.M. (Rotunda Hospital. 2n. Ed. Pp. 1-184. Illustrated. Philosophical Library. New York. N. Y.

This pocket-size volume is an excellent guide for nurses in the tropics and well fulfills the aim of the author to provide a practical and authoritative work to place in the hands of travelers in tropical regions. After a brief Introduction the book is divided into the following sections: Personal Hygiene in the Tropics, Diseases, Technique, Care of the Eyes, and a Glossary. The section upon disease encountered in the tropics considers all of the important diseases met with in such localities in a brief but comprehensive manner and embodies all of the essential facts which would prove to be useful to nurses or travelers and the data given are correct and up-to-date in most respects. The section devoted to Technique includes the technique of blood examinations, blood transfusion, bowel lavage, test meals and intramuscular and intravenous injections. There are also included a useful glossary and tables of weights and measures.

The book can be recommended to those for whom it is intended as a reliable and useful one and deserving of a wide circulation.

CHAS. F. CRAIG.



## WARRINGTON YORKE MEMORIAL FUND

The late Professor Warrington Yorke was a product of the Liverpool School of Tropical Medicine, and one of its most distinguished members. In addition to his jealous maintenance of the high standards set by earlier workers at the school, he earned for himself an international reputation in the world of medical science, and his outstanding original work on trypanosomiasis, blackwater fever, the nematode parasites, and many other parasitic and tropical diseases has permanently enriched our knowledge of these subjects.

In the latter part of his career, so untimely cut short, Yorke's exceptional energy and ability were increasingly devoted to the elucidation of the mode of action and the therapeutic value of chemical compounds, especially in parasitic diseases. As a direct result of his pioneer work, new and more potent weapons were forged to combat a number of diseases, in particular leishmaniasis and trypanosomiasis. That these discoveries were of far more than academic interest has been proved by their increasingly wide employment; indeed, it may be said that Yorke's introduction of drugs of the diamidine series is rendering possible the mastery of kala-azar in those parts of the world where the disease is peculiarly resistant to the antimonial compounds.

At the time of his death further studies in chemotherapy had been initiated by him, and it was his avowed object to promote chemotherapeutic research in Great Britain to the front rank and firmly to establish Liverpool as one of its leading centres. To that end, he laid a sound and solid foundation on which to build, but, though he lived long enough to see the realization of his ambition begun, he did not see it consolidated.

The Council of the Liverpool School of Tropical Medicine feels that a fitting memorial to this remarkable man would be to place on a firm financial basis the recently created Chemotherapeutic Research Department, where the work which he inaugurated will be continued in association with his name. To this end a Warrington Yorke Memorial Fund has been opened, and the Council believes that Yorke's many colleagues, friends, past students and others who have benefited by the great advances which he helped to make in tropical medicine and hygiene during his forty years of service, will wish to be associated with this memorial; from such the Council would welcome subscriptions, however small, which should be addressed to:—The Honorary Treasurer, Liverpool School of Tropical Medicine, The Chamber of Commerce, 1, Old Hall Street, Liverpool.





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*Editor, CHAS. F. CRAIG*  
Colonel, U. S. Army, Retired  
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## SEROLOGICAL STUDIES OF BULLIS FEVER

H. R. LIVESAY AND M. POLLARD<sup>1</sup>

The clinical syndrome which is referred to as Bullis fever (1) and which is associated with a rickettsia-like agent (2) was further studied for serological identification or differentiation from some of the known rickettsial agents. The lack of relationship existing between the agent of Bullis fever and that of Rocky Mountain Spotted fever has been demonstrated by guinea pig challenge experiments (2). There has been some slight suggestion of similarity of the clinical picture to that of Q fever. This is not confirmed in the following report.

The majority of cases occurred in one (1) group of men, all stationed simultaneously at Camp Bullis, Texas, and coincidentally during a very high degree of insect infestation of the area. Two (2) months after the cessation of the outbreak, two hundred and sixty-one (261) serums were collected from cases which were diagnosed clinically as the Bullis fever syndrome. All of these cases gave a history of tick<sup>2</sup> and chigger bites, sudden onset of illness, severe headache, lymphadenopathy, and leukopenia. All specimens of serum were collected aseptically in vacuum tubes and were transported immediately to the laboratory. The serum was stored without preservative in sterile vials at 4°C. until examined. In addition, specimens of serum were similarly collected from cases which occurred during this period in the personnel of other units; also from cases of unrelated diseases and normal individuals as controls.

Several species of animals from the Camp Bullis area were shot and serum specimens were collected for examination: this included 40 deer (*Odocoileus virginianus* subsp.), 7 rabbits (*Lepus californicus* subsp.), 2 raccoons (*Procyon lotor fuscipes*), and 1 armadillo (*Dasypus novemcinctus*).

All serum specimens were tested for Weil-Felix agglutination reaction and by the complement fixation reaction for endemic typhus, American Q fever and Bullis fever (one hundred and ninety-two (192) human and fifty (50) animal specimens for the latter disease). Fifty-six (56) of the human

and 14 of the deer specimens were kindly examined for Q fever by Mr. D. M. Wolfe of the Rocky Mountain Laboratory, Hamilton, Montana, using Q. F. Rickettsia agglutination and complement fixation tests.

The complement-fixing antigens for Q fever and endemic typhus were prepared from the infected chick embryo yolk sac. The Bullis fever antigen was prepared by triturating with sand the enlarged infected spleens of mice<sup>3</sup> and adding approximately twenty (20) per cent by volume sterile physiological saline. The heavy material was permitted to settle out at 4°C. overnight or by light centrifugation for five (5) minutes at one thousand (1000) r.p.m. The supernatant in either case was used as antigen. This was titrated before use with human serum from which the agent was isolated. Unfortunately, an insufficient quantity of the latter antigen was prepared to titrate the serums to their end points. This was prevented by the sudden disappearance of the agent from our experimental animals.

The conventional complement fixation test was set up with 1:5 and 1:10 dilutions of inactivated serum, two (2) units of antigen, and two (2) units of complement. Following a primary incubation period of fifty (50) minutes at 37°C., the amboceptor and cells were added. The tests were read after a second incubation at 37°C. for forty-five (45) minutes.

As indicated in chart 1, fifty (50) random cases are tabulated. All gave negative Weil-Felix reactions. All were negative for endemic typhus and two (2) cases were positive for American Q fever. Forty-seven (83.9%) gave positive results for Bullis fever: thirty-nine (39) with 4+ and eight (8) with 2+ fixation. Of the two (2) positive Q fever cases, one (1) was negative and one (1) was positive for Bullis fever. One was positive by serum neutralization for Rocky Mountain Spotted fever. Of the remaining one hundred and forty-two (142) human serums examined for Bullis fever, one hundred (70.5%) gave positive results for Bullis fever: seventy-six (76) were 4+ positive and twenty-four (24) were 2+ positive. The re-

<sup>1</sup> Colonel, Medical Corps, United States Army, and Captain, Veterinary Corps, Army of United States.

<sup>2</sup> Presumably *Amblyomma americanum* because of their predominance in the area.

<sup>3</sup> Infected with A.M. strain, isolated from a human case of the disease.

CHART 1  
Agglutination Ox—Weil-Felix Reaction

NO.	NAME	AGGLUTINATION OX			T.F. C-F	Q.F. AGGLUT.	Q.F. C-F	B.F.	C-F
		19	2	K	1:5	1:5	1:5	1:5	1:10
1	J. M.	1:10	1:20	—	—	—	—	4+	2+
2	J. D.	—	—	—	—	—	—	4+	2+
3	T. N.	1:10	1:10	—	—	—	—	4+	3+
4	W. D.	—	—	—	—	—	—	4+	2+
5	E. B.	1:20	—	1:10	—	—	—	2+	—
6	A. G.	—	—	—	—	—	—	4+	4+
7	J. M.	—	—	1:20	—	—	—	4+	4+
8	G. L.	1:10	1:10	—	—	—	—	1+	—
9	J. M.	—	—	—	—	—	—	3+	—
10	C. E.	1:80	1:40	1:20	—	—	—	4+	3+
11	H. B.	1:20	—	1:10	—	—	—	4+	3+
12	J. R.	1:20	—	1:20	—	—	—	4+	4+
13	W. R.	—	—	—	—	—	—	2+	1+
14	J. M.	1:40	—	—	—	—	—	4+	2+
15	R. O.	1:10	—	—	—	—	—	4+	3+
16	A. P.	1:20	—	—	—	—	—	4+	4+
17	P. D.	1:10	1:10	1:40	—	—	—	4+	1+
18	M. K.	1:10	—	—	—	—	—	3+	1+
19	A. A.	1:20	—	—	—	—	—	1+	—
20	J. S.	—	—	—	—	—	—	—	—
21	O. K.	1:40	—	1:10	—	—	—	4+	4+
22	T. C.	—	—	—	—	—	—	4+	4+
23	H. K.	1:10	—	—	—	—	—	4+	3+
24	A. A.	1:10	—	—	—	—	—	2+	—
25	G. L.	—	—	—	—	—	—	4+	2+
26	L. H.	1:20	—	—	—	—	—	4+	4+
27	J. A.	—	—	—	—	—	—	1+	—
28	D. R.	—	1:10	—	—	—	—	4+	3+
29	J. C.	1:10	1:10	—	—	—	—	4+	4+
30	T. W.	—	—	—	—	—	—	3+	—
31	J. B.	1:10	—	—	—	—	—	4+	3+
32	W. K.	—	—	—	—	—	—	4+	4+
33	C. B.	—	—	—	—	—	—	4+	4+
34	J. S.	—	—	—	—	—	—	4+	—
35	J.	—	—	—	—	—	—	2+	—
36	E. N.	—	1:10	—	—	—	—	4+	1+
37	R. T.	—	1:10	—	—	—	—	4+	4+
38	G. A. S.	—	1:10	—	—	—	—	3+	—
39	T. R.	—	1:20	—	—	—	—	1+	—
40	J. E. B.	—	—	—	—	—	4 + 1:64 2 + 1:128	—	—
41	L. H.	—	—	—	—	—	—	3+	—
42	W. B.	—	—	—	—	—	—	4+	3+
43	A. F.	—	—	1:10	—	—	—	2+	—
44	W. D.	—	—	—	—	—	—	4+	2+
45	J. B.	—	—	—	—	—	—	4+	4+
46	L. L.	—	—	—	—	—	—	—	—
47	E. S.	1:10	—	—	—	—	4 + 1:16	4+	1+
48	N. B.	—	—	1:10	—	—	—	4+	—
49	P. L.	—	—	—	—	—	—	4+	4+
50	M. H.*	—	—	—	—	—	—	—	—

T.F. C-F.—typhus fever complement fixation; Q.F. agglut.—Q fever rickettsial agglutination; Q.F. C-F.—Q fever complement fixation; B.F. C-F.—Bullis fever complement fixation.

\* Positive for Rocky Mountain Spotted Fever.

maintaining forty-two (42) were negative. All tests were negative for endemic typhus, and Weil-Felix reaction. As was indicated in a previous report (2), the Weil-Felix reaction remained significantly negative during the entire acute stage of the disease and during convalescence.

Of forty (40) deer specimens, four (4) exhibited positive complement fixation for Bullis fever, and two (2) others for Q fever. Two (2) of the seven (7) rabbits were also positive for Bullis fever and all were negative for Q fever. The raccoons were negative for both Bullis fever and Q fever. The one (1) armadillo examined was anticomplementary. All of the animal serums examined were negative for endemic typhus and significant Weil-Felix reactions.

evidence of this disease; however, enough of them were positive to permit us to attach some significance to the results. Seventy-six (76) per cent of the one hundred and ninety-two (192) specimens gave positive complement fixation for this disease: 78.2% of these with strong positive, and 21.8% with strongly suspicious reactions. The etiology of those cases with negative serology for Bullis fever must necessarily be left to conjecture: with some, the serological sensitivity may have disappeared by the time the specimens were collected; for the rest, other unidentified etiological agents may be responsible for the disturbance.

The specificity of the antigen was supported by the negative reactions with serum from cases of endemic typhus fever, Q fever, Rocky Mountain

CHART 2

SPECIMENS	BULLIS FEVER	%	TYPHUS	%	Q FEVER	%
261 Bullis fever positive.....	147/192*	76.5	0/261	0	2/261	0.76
40 Deer positive.....	4/40	10	0/261	0	2/40	5†
7 Rabbits.....	2/7	28.5	0/7	0	0/7	0
2 Raccoon controls.....	0/2	0	0/2	0	0/2	0
29 Normal human.....	0/29	0	0/29	0	0/29	0
4 Typhus fever.....	0/4	0	4/4	100	0/4	0
3 Spotted fever.....	0/4	0	0/4	0	0/4	0
4 Convalescent scrub typhus.....	0/4	0	0/4	0	0/4	0
2 Q fever.....	0/2	0	0/2	0	2/2	100

\* Denominator represents the number examined; numerator is the number of positives.

† Not the same as those positive for Bullis Fever.

Control serum specimens from heterologous infections which were negative for Bullis fever were as follows: four (4) cases of endemic typhus, three (3) cases of Rocky Mountain Spotted fever, two (2) cases of Q fever, and four (4) cases of scrub typhus. In addition, twenty-nine (29) normal serums were tested for Bullis fever, Q fever, endemic typhus, and Weil-Felix reaction with negative results.

In order to determine the possible influence of the heterophile antibody with the mouse spleen antigen for Bullis fever, some of the positive and negative serums were examined for this antibody. In no instance was a significant heterophile antibody titer obtained: none was above 1:20.

#### DISCUSSION

Not all of the cases which were designated as Bullis fever clinically gave positive serological

Spotted fever, scrub typhus and twenty-nine (29) normal serums. The bizarre serology in some cases of Q fever requires some conservatism as to the interpretation of the results of examinations for this disease.

The positive serology for Bullis fever in four (4) of forty (40) deer and two (2) of seven (7) rabbits might mean that these animals constitute the reservoir species, or more probably that these animals are showing evidence of past exposure to the disease agent. Until the active agent can be demonstrated in the suspected species, it is illogical to call them reservoir hosts. It is conceded, however, that the animals contribute to the propagation of those insects which may transmit the disease. An agent has been isolated from Camp Bullis ticks (*A. americanum*) (3), which seems to bear some similarity to the agent isolated by us from a human case of the disease.



## CONCLUSIONS

1. The Bullis fever syndrome is not characterized by a significant *Proteus* OX-19, OX-K, or OX-2 agglutination reaction.

2. There does not appear to be any serological relationship between the rickettsia-like agent of Bullis fever and the rickettsia of American Q fever by the complement fixation test.

3. From the results obtained, there does appear to be some significant serological relationship between the agent isolated from a human case of Bullis fever and the serums of convalescent cases.

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# EARLY FILARIASIS DIAGNOSIS AND CLINICAL FINDINGS: A REPORT OF 268 CASES IN AMERICAN TROOPS

BOYD G. KING<sup>1</sup>

*From the Medical Service of the Fourth General Hospital*

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## INTRODUCTION

The early lesions produced by infection with *Wuchereria bancrofti* have been described in the past by several observers (6, 2, 16, 17, 24). These have usually occurred in native populations in whom it was difficult to define the earliest manifestations. We have recently observed 268 American troops who had lived an average of four months in Pacific islands and who presented symptoms and signs which we believe were due to filariasis. Most of the patients had never lived in the tropics before, so that the lesions found represent the earliest manifestations of the disease. Our observations began eight months after the patients had first arrived in the endemic areas and in the majority of instances within a few days or weeks after the first symptoms. Our attention was first drawn to the possibility of filariasis in this group by a patient who had epididymitis and then developed lymphangitis of one arm. This led to biopsy of a lymph node in another patient, sections of which showed adult filariae in the subcapsular sinuses. In the succeeding eight months the present series was observed.

The life cycle of *Wuchereria bancrofti* is well known and need not be discussed here. It normally inhabits the lymphatic system of man, giving rise to a host of clinical conditions as it progressively produces pathological change. Lymphadenitis and lymphangitis have been considered by others (2, 6) to be the early lesions of the pathology of filariasis and our experience supports this view. This paper deals with the clinical aspect of the 268 cases. The pathology is separately reported by Wartman (26).

## SOURCE OF INFECTION

229 (85.4%) of this group had spent one month on one island, two and a half months on another and two weeks on a third. 31 (11.6%) had spent four months on the third island only. The remaining 8 (3%) had lived variously from two to eleven months in the above areas. This was in the period

from the first part of May, 1942 until September, 1942. We did not come into contact with the group until January, 1943. No cases were seen who had not been in the above-mentioned areas. By far the larger number of patients had spent two and a half months on Island No. 2. All the men lived in or near the jungle and of necessity in close proximity to the natives. The recent incidence of filariasis in the natives on Island 3 is not known to us. However, from personal communications, many of them were observed to have elephantiasis and/or microfilariae in the peripheral blood. Approximately one fifth of the troops stationed there were admitted to this hospital with manifestations of filariasis.

## INCUBATION PERIOD

The onset of the disease was designated as the date of the first clinical symptoms, which were carefully evaluated in taking the histories. If there was doubt as to the character of the symptoms, the date of onset was noted to be that of the date of onset of the presenting complaint in the hospital. Our observations began eight months after the first possible contact. It is known (personal communications) that clinical cases similar to ours were observed in this group as early as October, 1942 (five months after exposure), so that we have no reason to doubt the validity of the histories given us. In this regard the case of O'Connor's assistant (2) who developed epitrochlear adenitis 43 days after being bitten by filariated mosquitoes is pertinent.

The earliest onset was three months after arrival in the islands (fig. 1). During the period during which we had contact with the troops, there was a rather constant incidence of onset of the disease from month to month. The troops were removed from our area, so the longest interval before symptoms occur is as yet unknown. The relatively slight variations in incidence from the tenth to the fifteenth months would lead to the belief that further cases would continue to appear either in similar or in decreasing numbers for an indefinite period. The slight increase in the fifteenth month

<sup>1</sup> Major, Med. Corps, Army of the United States.

was due to a concerted effort to find all the cases in the group for proper disposition. No estimate of average incubation period of any validity is possible from this group, but it is fairly certain not to occur before three months exposure, and the increasing incidence thereafter becomes constant at about the tenth month.

#### MODE OF ONSET

Almost none of the patients had more than a mild constitutional reaction at the onset of symptoms. In only two instances did complaints include chills, fever, malaise and headache. The first symptoms were largely local, consisting of pain, swelling or redness of an arm or leg; or pain and swelling in the scrotal region. The pain in

that the tabulation is largely from the patients' histories. Actual findings are given below.

#### CLINICAL SIGNS AND SYMPTOMS

Of the various filarial disturbances, we observed no cases of arthritis, hip joint abscess, varicose groin or axillary glands, lymph scrotum, chyluria, elephantiasis or chylous hydrocele, chylous diarrhea or chylous ascites. There was one doubtful case of synovitis of the knee not included in this series. In another there was an abscess apparently arising in the femoral glands, which yielded *Staphylococcus aureus* on culture, and in which there was a positive intradermal reaction to *D. immitis* antigen (see below). There was, however, no other clinical evidence of filariasis.

#### Ex. Cases

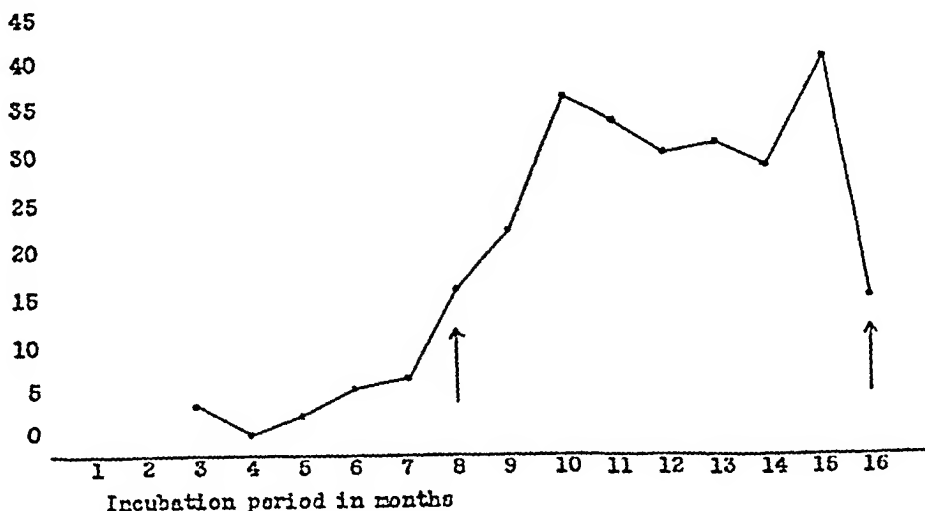


FIG. 1. ↑—↑: PERIOD DURING WHICH THE PATIENTS WERE OBSERVED

the extremities was never severe, but usually accompanied by stiffness. The swelling was most often limited to the medial surface of the upper arm, or the anterior surface of the forearm, and when redness was noted it was usually diffuse in the same areas or in the form of a streak over the area of swelling. The swelling of the scrotum and its contents was more uncomfortable, but still not often severe. In the few instances of onset in the leg, an occasional patient mentioned mild generalized swelling below the knee. Pain in the groin was not uncommonly the first symptom, while pain in either lower abdominal quadrant occurred twice. In five instances arm and genital symptoms occurred simultaneously. The site and type of onset are shown in table 1. It should be borne in mind

The syndromes observed fall roughly into three categories: lymphangitis of extremity or trunk, acute inflammation of the scrotum or its contents, and lymph node enlargement. Fever was recorded in 53 (19.7%) of the 268 cases. In all but a few instances it was very mild, reaching 99 or 100 F. for a few days, occasionally prolonged up to as long as six weeks. In four instances there was fever up to 102 or 103 F. for from two to six days, associated with lymphangitis. In one instance the fever reached 104 F. at onset and fell by lysis over a period of 12 days, with no local symptoms until the sixth day, when transient epididymitis occurred. This case simulated paratyphoid fever but no evidence for it could be found.

The relapsing nature of the symptoms as de-

scribed by many (17, 24) was amply verified in our experience. Lymphangitis occurred as many as six times in one individual, and many of the cases had had recurrences of either subcutaneous or genital inflammation one or more times. In general the acute local symptoms were of short duration, rarely persisting over ten days, and often disappearing within two or three days. A striking feature of the whole group was the lack of severe constitutional symptoms. The patients did not

uation facilities some patients had to be discharged before signs were completely subsided and others had slightly prolonged stay. Consequently the above figure is an approximation of the amount of time lost for each attack, but it is believed that it is not far wrong in the average case. Seventeen patients were sent to duty because of the apparent minimal nature of the symptoms or because of doubt in the diagnosis. Twelve of these returned for a second admission at which time the diagnosis

TABLE 1  
*Mode of Onset*

SITE AND SYMPTOMS OF FIRST LESION	NO. CASES	%	NO. WITH SYMPTOMS	%
I. Genitalia.....	152	55.5		
Swelling.....			134	88.2
Pain in testis.....			125	82.2
Pain in groin.....			15	9.8
Redness of scrotum.....			2	1.3
II. Arm.....	105	38.3		
Swelling.....			92	87.6
Pain.....			82	78.1
Redness.....			50	47.6
Pruritus.....			3	2.9
III. Leg.....	10	3.6		
Pain.....			8	80.0
Swelling.....			7	70.0
Redness.....			2	20.0
IV. Miscellaneous.....	7	2.6		
Chill, fever, malaise.....			2	
Pain, swelling in flank.....			1	
Pain right lower quadrant.....			1	
Pain left lower quadrant.....			1	
Pain, swelling in neck.....			1	
V. Combined sites at onset.....				
Genitalia and arm.....	5			
Arm and leg.....	1			

feel sick, and the local lesions were not extraordinarily painful or disabling. In no instance did there seem to be danger to life. However, the disease was most important from the military standpoint because the lesions were incompatible with full field duty. The aches and pains of the extremities or testicles were definitely aggravated by exertion, and the tendency to recurrences placed the man's future efficiency in doubt.

After allowing for concurrent illnesses, the average hospital stay was 15.9 days. Because of evac-

was more evident. The remaining 5 did not return to this hospital.

#### *Lymphangitis*

Inflammation of the lymphatics involving the arm, leg or trunk is known to be common in filarial diseases. It was observed in 109 cases in this series. In an additional 28 cases there was a history of lymphangitis making a total of 137, an incidence of 51.1%. Table 2 shows the location of the lesions in the body. The predominance

of arm lesions correlates with the high incidence of epitrochlear adenitis to be described below.

The lymphangitis of the arm occurred most commonly along a course overlying the brachial vessels or on the volar surface of the forearm. It took three forms: A red streak of varying length often with an underlying firm irregular cord, most common in the upper arm; patches of subcutaneous edema and overlying redness, irregular in outline and of varying size and most common on the anterior surface of the forearm; and diffuse edema and erythema of the upper arm or forearm. With the erythema there was increased local heat. Tenderness was mild or moderate, rarely severe. The

TABLE 2

*Site of Lymphangitis (Excluding Genitalia)*

I. Arm.....	124	
Unilateral.....		91
Bilateral.....		7
History of lymphangitis....		26
II. Leg.....	20	
Unilateral.....		18
Bilateral.....		0
History of lymphangitis....		2
III. Trunk.....	5	
Neck.....		3
Groin and buttock.....		2
Total times occurred.....	149	
IV. Combined involvement.....	12	
Arm and leg.....		7
Extremity and trunk.....		5
Total no. individuals.....	137 (51.1% of 268 cases)	

streaks were often multiple and in one case there was a graphic anatomical demonstration of all the superficial lymphatics of both the upper and the lower arm outlined by the inflammatory reaction. The irregular cords, formed along the lymphatics, persisted after the acute inflammation subsided, but over a period of a few days, tended to disappear. Residually there were at times poorly-defined areas of subcutaneous induration or nodules which felt like lymph nodes. In other instances there were only patches of subcutaneous edema, of a peculiar "blubbery" consistency, with no erythema or tenderness, and which tended to persist for two or three weeks.

In the leg the lymphangitis was usually along the medial surface of the thigh, and in only four instances was there a definite streak. Patches of swelling in the popliteal area, the calf or about the ankle were observed. In three instances transient areas of lymphangitis occurred on the side of the neck and in one, over an eyebrow. There were two cases of a wide (3 cm.) streak of lymphangitis starting in the groin and extending laterally about the hip nearly to the intergluteal fold.

Perhaps the most unusual feature of the lymphangitis was its tendency to extend peripherally. It often appeared in a limited area and did not spread, but it was observed in many instances to spread *down* the arm, or centrifugally. The two cases starting in the groin spread *away* from the inguinal nodes. This feature was not observed in the legs. Some of the patients spontaneously described this spread in giving the histories of their symptoms. Fever and malaise were irregularly present and were not prominent. Leucocytosis was usually only mild if present at all. Local adenopathy often accompanied the lymphangitis, but was not invariably present. Urticaria of local distribution over areas of lymphangitis occurred rarely. In a few instances, after the acute erythema had subsided, ecchymotic discoloration of the skin was present, usually overlying a subcutaneous cord-like structure.

### *Genital Lesions*

Inflammation of the spermatic cord, epididymis, testicle or scrotum, or a combined involvement of more than one of the intrascrotal structures was the most common lesion observed, occurring in 192 (71.6%) individuals, of which 183 were seen by us, the remaining 9 giving a history of testicular swelling. The usual history was of a painful swelling of the testicle, which may have been intermittent for weeks or months, but usually was seen by us at or soon after the first attack.

Table 3 shows the various lesions encountered. Funiculitis and epididymitis was the most frequent combination observed. The swelling usually involved the spermatic cord from the external inguinal ring to its junction with the epididymis and swelling of the two structures was nearly always concurrent. It was sometimes only a slight thickening of one side as compared with the other. At other times there was marked edema, usually rather rubbery in consistency. The swelling tended to subside in a few days. The glans major of the

epididymis was most frequently involved, extending into the body and globus minor only infrequently. The presence of palpable nodules in the epididymis and less frequently in the cord is noted in table 3. These were usually quite firm but occasionally were "blubbery" or cystic in consistency. In most instances the lesion subsided completely; in others there remained palpable thickening or nodularity of the epididymis or cord or both.

The testicle itself was less frequently swollen, in 25 cases only. Occasionally all intrascrotal structures were swollen and indurated and it was impossible to differentiate them. Edema and erythema of the scrotal skin was observed 34 times and usually subsided very quickly. In one case the scrotal inflammation extended upwards as a

TABLE 3  
*Genital Lymphangitis*

	NO.	%
History of scrotal swelling.....	9	
Cases observed.....	183	
Total no. of cases.....	192	71.6
Lesions found in cases observed:		
Epididymitis.....	171	93.4
Funiculitis.....	124	67.7
Scrotal inflammation.....	34	18.5
Orchitis.....	25	13.7
Hydrocele.....	10	5.5
Bilateral lesions.....	19	10.4
Nodular lesions.....	21	11.5

streak of lymphangitis ending at the umbilicus. Ten small hydroceles were encountered, none of which required therapeutic aspiration, and all subsided before discharge from the hospital.

Pain and tenderness of the genital lesions were more marked than in the lymphangitis of the extremities and were at times exquisite. One not uncommon feature was tenderness over the inguinal canal in association with swelling of the spermatic cord below the external inguinal ring. There were a few instances of edema over the inguinal canal extending up to the internal ring. The discomfort was aggravated by walking or jarring as would seem inevitable. Genital lesions occurred bilaterally in 19 cases. These findings correspond closely with the early lesions reported by Knott (13) and Ferrer (10).

### *Lymphadenopathy*

Table 4 lists the various areas of the body in which enlargement of lymph nodes was observed. This enlargement was determined by clinical examination done in the main by three observers. It was found difficult to be positive as to the presence or absence of enlargement when the deviation from the normal was minimal. This was particularly true in the inguinal region where so many individuals have readily palpable small nodes. It was easiest in the epitrochlear region where normally the nodes are barely palpable, if at all. Many records of the patients state that

TABLE 4  
*Lymph Node Enlargement*

	NO. x FOUND	%
Epitrochlear region.....	143	53.3
Bilateral.....	61	
Unilateral.....	82	
Axillary region.....	97	36.2
Bilateral.....	47	
Unilateral.....	50	
Inguinal region.....	95	35.4
Bilateral.....	61	
Unilateral.....	34	
Femoral region.....	105	39.2
Bilateral.....	68	
Unilateral.....	37	
Cervical region.....	1	
Supraclavicular region.....	2	
Total no. cases with some degree of lymph node enlargement.....	228	85.1

the femoral and inguinal nodes "are palpable." These were not counted as enlarged in the final tabulation. The frequency with which the adenopathy was bilateral in the inguinal and femoral regions as contrasted to the infrequency of lymphangitis in the legs would suggest a general lymphatic hyperplasia as being a part of the pathology of filarial infection. In 4 cases lymph node enlargement was the only manifestation of the disease either by history or observation in the hospital. In many more, we observed only lymph node enlargement but there was a history of lymphangitis or genital swelling before admission.

In the axillary region the node enlargement was usually not more than moderate, with an occasional very large node (3 x 3 cm.). Inguinal and femoral node enlargement was more often bilateral and tended to be moderate or less. When it was marked, tenderness was also present but subsided quickly. The most striking lymphadenopathy was in the epitrochlear region where 143 (53.3%) cases had enlarged glands. This is in agreement with the findings of others (2) in natives in the Pacific Islands. The nodes were always quite firm, usually single but some in clusters. They tended to form a chain beginning in the ordinary epitrochlear situation and extending up the medial surface of the arm, at times half the distance to the axilla. Isolated nodes in the mid-humeral region along the course of the brachial vein and in the antecubital fossa were found occasionally. The enlargement at times reached the size of a walnut but was ordinarily smaller. The first node to be removed for biopsy was in the antecubital space and proved to contain adult filariae. Rarely cervical and supraclavicular node enlargement was found.

The enlargement of all nodes tended to persist and in the few cases observed for a second time in the hospital the adenopathy was found to be unchanged or more extensive. No cases of so-called "varicose" glands were observed. Ectopic nodules were found in various parts of the wrist, forearm and arm, but these were probably not true nodes since they were usually the result of previous lymphangitis and tended to decrease slowly in size.

#### LABORATORY FINDINGS

Table 5 enumerates the laboratory data obtained on these patients. The white blood cell count was within the normal range (5,000-10,000 per cmm.) in two thirds of the cases, but was significantly elevated in nearly a third. This elevation was usually present without fever, and often tended to persist for several days and occasionally for a few weeks. There were also many instances of normal counts when fever was present.

The eosinophiles were elevated in approximately two thirds of the patients. They were invariably mature cells. There was no consistency in the relative numbers of lymphocytes and polymorphonuclear leucocytes, either of which were at times moderately increased or reduced. The figures given in table 5 represent a composite picture of the average eosinophilia in each patient, of whom

the majority had only one white blood cell count and differential estimation done. The eosinophiles are tabulated in absolute numbers as well as in percentages of the total count. The normal is considered to be 0-4% of 5,000-10,000 white blood cells (0-400 eosinophiles). It is shown that those patients with intestinal parasites tended to have slightly more eosinophiles than those without. There were several instances of 3,000 or more eosinophiles per cmm. (with or without intestinal parasites). The highest recorded was 17,419 eosinophiles per cmm. in a patient who in addition to lymphangitis had a chest film resembling early silicosis.

The stools were examined by the zinc sulfate floatation method. Most of the patients had a single examination on admission, but 67 had multiple examinations with negative results. As is shown in table 5, 66 (25%) had some variety of intestinal parasite, hookworm and whipworm predominating.

#### *Search for Microfilaria*

The results in this study are simply stated: None were found, even after diligent search. All patients were examined either by the staining of a thick blood smear, or by search of the formalinized sediment of 1 cc. of blood by Knott's method (14). At least half the series was examined by both methods. In the beginning various techniques were used: Laking 1-10 cc. of blood with 10% saponin solution or 2% acetic acid solution and examining the centrifuged sediment before and after staining, and examination of fresh preparations under a cover slip or as a hanging drop. Blood from seven typical cases, two of them with biopsies showing adult female filariae in lymphatic tissue was drawn at four different hours of the day and night on four different days. A total of over 60 examinations was done on these seven patients with negative results. Thick blood smear stained with methylene blue and eosin was used routinely at first, but Knott's method was used in the last 100 cases. Search was made at various times of the day, and in the first 150 cases was done twice on each patient, at noon and midnight. More recently a single examination in the early evening has been routine, since the Pacific variety of *W. bancrofti* is known to have non-periodic microfilariae (16).

In addition to the search on the patients, 46 men from the battalion most heavily infected, and who

had positive intradermal reactions (see below) were examined by Knott's method and by the thick smear technique with negative results. Ninety-eight men of a small unit who had spent 6-11 months on one island, 30 of them for five months on the second island, were examined by Knott's method with negative results. To summarize, in

### Miscellaneous Findings

Urinalysis, both chemical and microscopic, was done on each patient. Those exhibiting pyuria were searched for lower urinary and genital tract infection by competent urologists. The Kahn reaction was done in 26 instances all of which were

TABLE 5  
Laboratory Findings

		%	
White blood cell counts:			
5000 or less.....		7 (2.3)	
5000-10000.....		209 (67.8)	
10000-15000.....		83 (26.9)	
15000 or over.....		9 (3.0)	
Total done on 268 cases.....		308 (100.0%)	
		% OF WBC COUNT	TOTAL PER CMM.
Eosinophiles (based on individual averages of 266 cases):			
Average for entire group.....		8.5	847
Average for 65 with intest. parasites.....		10.3	1029
Average for 201 without intest. parasites.....		8.0	793
		NO. CASES	RANGE
Range of eosinophilia:		%	
A. Total per cmm.			
Normal.....		95 (35.7)	0-400
Moderate elevation.....		105 (39.5)	400-1000
Marked elevation.....		66 (24.8)	1000
B. Percentage of total WBC count			
Normal.....		93 (35.0)	0-4%
Moderate elevation.....		150 (56.4)	5-19%
Marked elevation.....		23 (8.6)	20-69%
Intestinal parasites (264 cases studied):			
Hookworm ( <i>N. americanus</i> ).....		37	
Whipworm ( <i>T. trichiura</i> ).....		19	
Hookworm and whipworm.....		5	
Dwarf tapeworm ( <i>H. nana</i> ).....		2	
<i>A. lumbricoides</i> .....		1	
Whipworm and <i>A. lumbricoides</i> .....		1	
Pinworm ( <i>E. vermicularis</i> ).....		1	
Total.....		66 (25%)	

addition to the 268 cases in this series, 154 men who had lived in an endemic area for from four to eleven months were found not to have microfilariae in the blood. The search was made from nine to sixteen months after the men had arrived in the endemic areas. This is a recorded total of 422 individuals, which added to numerous examinations on unrecorded cases, would approach 500.

negative. Sedimentation rate was normal in 6 and slightly elevated in 2. The heterophile antibody reaction was within normal limits in the only 4 examinations done. Two examinations of aspirated hydrocele fluid failed to reveal microfilariae, although one contained many eosinophiles. In 9 selected cases with definite lymphangitis or adenopathy in the epitrochlear region, soft tissue



X-ray films showed no calcification. In 5 cases of epididymitis and funiculitis, X-rays of the scrotum also failed to show calcific deposit.

#### DIAGNOSIS

The positive diagnosis of filarial infection can be made by three methods: The finding of adult or larval filariae in tissue obtained at biopsy or post-mortem examination, the finding of microfilariae in the blood, or the finding of calcified worms by X-ray technique (11, 21). As has been stated no microfilariae in the blood or calcified worms on the X-ray films were found. The biopsy material is reported by Wartman (26).

We believe the diagnosis can be established clinically with very little doubt. The history of prolonged stay in an endemic area; the finding of lymphangitis of extremity, trunk or genitalia coming on after an interval of at least three months; combined with adenopathy, eosinophilia and a positive intradermal reaction completes the characteristic picture of early filariasis. The diagnosis has not been absolutely proven in all of our cases, but no other cause for the syndromes presented could be adequately supported.

The lymphangitis of trunk or extremity did not conform with the clinical findings of bacterial infection. The many instances without fever or leucocytosis, the infrequency of malaise, the centrifugal spread of many of the lesions, the absence of suppuration and the sterile cultures of biopsy material (26) oppose bacterial infection as the cause. This corresponds with O'Connor's conclusions (19) and the prevailing opinion at present. However, the confusion of this lesion with thrombophlebitis is not uncommon. The streak of erythema with underlying cord-like induration over the distribution of a vein may almost perfectly simulate venous inflammation. In at least two of our cases, thrombophlebitis of the brachial vein was the initial diagnosis, but the patients later returned with characteristic lymphangitis of filariasis.

Syphilis, tuberculosis, Hodgkin's disease and all other causes for lymphadenopathy must be ruled out. Nothing suggesting other causes was found in this group. The predominance of involvement of the epitrochlear nodes and the percentage incidence of each group of nodes involved compares closely with Buxton's observations on natives in the Pacific Islands (2).

The differential diagnosis of the genital lesions can be difficult. Ferrer believes that filariasis can

be diagnosed on clinical grounds without microfilariae in the superficial circulation (10). Knott's findings in early filariasis correspond closely with our own (13). Non-gonorrheal epididymitis secondary to lower urinary tract infection was eliminated by examination of the prostate and seminal vesicles in all cases with a history of urethral discharge or with pyuria. Gonorrheal epididymitis was eliminated by careful examination by the urological service when it seemed indicated. The filarial lesion is in contrast to the very hard, diffuse, exquisitely tender swelling seen secondary to gonorrheal infection. Tuberculous epididymitis tends to be more insidious, progressive, and associated with prostatic infection, "beading" of the vas deferens and not infrequent suppuration. Concomitant lymphadenopathy or lymphangitis of an extremity aids in the diagnosis. Early filarial hydrocele in the absence of other findings may be impossible to distinguish from other inflammatory hydroceles.

Filariasis of the spermatic cord has been mistaken for hernia (10). This error was made by us in one instance. Operation revealed a mass of inflammatory tissue in the right inguinal canal and no hernial sac. Biopsy of this tissue proved it to be of a chronic granulomatous nature, suggestive of filariasis. Two weeks after operation the right spermatic cord and epididymis became swollen and a week later the left spermatic cord was similarly involved and seemed typically filarial.

Pain in the lower abdomen can be confusing and usually precedes any demonstrable lesion. In one case, three days following the removal of a normal appendix for what had seemed to be atypical appendicitis, the right spermatic cord and epididymis were acutely swollen and epitrochlear adenopathy was observed. No cause other than filariasis could be found. We have not had opportunity to observe retroperitoneal lymphatic involvement, but it is said to occur and produce fever and abdominal pain (17).

#### Intradermal Reactions

The intradermal reaction as originally described by Taliaferro and Hoffman (25) and by Fairley (9) was used as an aid in diagnosis. The antigen was prepared as described by Fairley. Specimens of the dog heart worm, *Dirofilaria immitis*, were dried and ground to a powder in a mortar. At first it was difficult to obtain consistent reactions, therefore two antigens, differing only slightly in the

technique of preparation were used on the patients. Had the difference in technique been discovered earlier only the "B" antigen described below would have been used routinely:

Antigen "A": The powdered *Dirofilaria* was weighed and enough physiologic saline solution added to make a 0.1% solution. This was incubated at 37°C. for two hours with frequent shaking, filtered through paper, then through a Seitz filter for sterility and placed in rubber-capped vials. This was the weaker antigen.

Antigen "B": The powdered *Dirofilaria* was weighed and enough physiologic saline solution added to make a 1% solution. This was incubated at 37°C. for two hours with frequent shaking, filtered through paper, then through a Seitz filter for sterility, and placed in rubber-capped vials. Before use this was diluted to 0.1% strength with more physiologic saline solution. This was the stronger antigen.

It is seen that the only difference in the two is the strength of the solution during extraction, "A" being 0.1% and "B" being 1% while incubating with the *Dirofilaria* powder. "B" was later diluted to 0.1% before use, so that the resultant strength of the two solutions should be equal. In fact they each contained 4 mgm. per 100 cc. of nitrogen, but the "B" antigen produced the more marked intradermal reactions. The latter also produced delayed reactions, whereas "A" antigen did not.

The skin test was done by injecting 0.1 cc. intradermally on the volar surface of the forearm. Control injection of 0.1 cc. of physiologic saline solution on the other arm was done in about the first 300 tests, but was then abandoned because none had produced a reaction comparable to a positive test with the antigen. Two types of reaction were observed, an immediate and a delayed. The *immediate* reaction was observed in 8-10 minutes and consisted of erythema, wheal and pseudopodia formation. Erythema alone was counted as 1 plus; wheal formation in addition counted as 2 plus; pseudopodia emanating from the wheal counted as 3 plus; and erythema greater than 5 cm. in diameter combined with wheal and pseudopodia was designated 4 plus. The *delayed* reaction was observed in 24 hours, although it may begin as early as four hours after the injection. It consists of erythema and subcutaneous edema, often associated with pruritus and mild aching. The edema had a rubbery consistency and did not pit on pressure. There was only mild tenderness. This type of reaction resembled closely the acute

lymphangitis seen in many of our patients, i.e., the diffuse variety without streaks or subcutaneous cords. An occasional patient reacted with edema of the entire arm and hand resembling acute cellulitis, which took from 3-7 days to subside completely. However, there were never any constitutional symptoms or fever. Interpretation of the delayed reactions was as follows: Erythema greater than 1.5 cm. in diameter counted as 1 plus; mild or moderate subcutaneous edema in addition counted as 2 plus; marked subcutaneous edema counted as 3 plus; and erythema with a diameter of more than 5 cm. combined with subcutaneous edema was designated as 4 plus.

The results of the intradermal reactions are given in table 6. No reaction was counted as positive in the final tabulation unless it was 2 plus or greater, considering either the immediate or the delayed reaction. It will be readily seen that "B" antigen gave the highest percentage of positive results in the patients, 90.8% as compared with 67.6% with "A" antigen. Further, the delayed reaction often seen with intradermal tests in parasitic infections (5) was produced only by the "B" antigen. This is a very significant difference in results with so little difference in technique of preparation, but it has been repeated with the same results on three different occasions.

The controls were done on individuals who had never spent time in the tropics. No one was used who had been in any of the Pacific Islands. Many of them had homes in the southern U. S. A. Some had spent vacations in the West Indies or Central America, but otherwise had not been known to be exposed to filarial infection. The number of controls used is comparable to the number of patients tested.

"A" antigen produced 97.4% negative results in the controls. "B" antigen produced 89.5% negative tests. A small group of controls were tested with a 0.5% antigen prepared exactly as the "B" solution, except that the final dilution was from 1% to 0.5% instead of to 0.1%. Out of 39 controls there were 28.2% positive results, with 18% delayed reactions. This indicated too great a number of false positive reactions and the 0.5% solution was abandoned for routine testing. Fairley (9) found that 1% extract produced severe delayed reactions and one case of acute anaphylaxis and abandoned it in favor of 0.1% solution thereafter. It would seem likely, therefore, that the stronger the reaction in patients the more false positive reactions produced, indicating that a high

percentage of individuals may be sensitive to the filarial protein if enough of it is introduced, but stance (0.6%) in the control series using the "B" antigen, but in 69 instances (42.1%) in the clinical

TABLE 6  
*Intradermal Reactions*

	NO. CASES	%
<b>"A" Antigen:</b>		
Positive (2 plus or greater).....	73	67.6
Negative (1 plus or less).....	35	32.4
Totals (No delayed reactions with "A" antigen).....	108	100.0
<b>"B" Antigen:</b>		
I. Positive (2 plus or greater).....	149	90.8
Negative (1 plus or less).....	15	9.2
Totals (This includes pos. immediate or delayed reactions or both)	164	100.0
II. Combinations of reactions:		
Pos. immediate, pos. delayed.....	65	39.6
Pos. immediate, neg. delayed.....	80	48.8
Neg. immediate, pos. delayed.....	4	2.4
Neg. immediate, neg. delayed.....	15	9.2
COMPARISON OF "A" and "B" ANTIGENS (41 cases tested with each):		
	"A"	"B"
Positive (immediate reaction).....	25	36
Negative (immediate reaction).....	16	5
Positive (delayed reaction).....	0	7
CONTROLS (Individuals unexposed to filariasis):		
	NO. CASES	%
<b>"A" Antigen</b>		
Positive (2 plus or greater).....	3	2.6
Negative (1 plus or less).....	111	97.4
Positive delayed reactions.....	0	0
Totals	114	100.0
<b>"B" Antigen</b>		
Positive (2 plus or greater).....	18	10.5
Negative (1 plus or less).....	153	89.5
Totals.....	171	100.0
Delayed reaction positive.....	1	0.6
0.5% Antigen (5 times strength of "B" antigen)		
Positive (2 plus or greater).....	11	28.2
Negative (1 plus or less).....	28	71.8
Totals.....	39	100.0
Delayed reaction positive.....	7	18.0

that patients with clinical filariasis will react to much smaller doses.

The delayed reaction was present in only 1 in-

cases of filariasis. This seems to us to probably be even more significant than the immediate wheal formation as indicative of filarial infestation.

However, only four times did it occur without a preceding positive immediate reaction, suggesting that the latter is the more sensitive indicator.

In contrast to the controls who had not been exposed to filariasis, 241 troops who had spent some months in Samoa and Wallis Island were tested. At the time they were tested only the "A" antigen was available. None of them had had clinical symptoms but some later developed clinical filariasis. Of this group 108 (44.8%) reacted positively to the antigen and no delayed reactions were seen. The incidence would probably have been higher if the "B" antigen had been used. These results suggest a high incidence of subclinical infection.

The possibility of other round worm infestations producing a cross-sensitivity has never been thoroughly investigated. We have been unable to clarify this problem entirely. It is well known that group reactions occur with helminth antigens (5, 9, 25). It has been thought that the *Dirofilaria immitis* antigen reaction is a group reaction common to all filarial diseases (5, 9) and this has been found to be true in a small number of reported investigations (3, 9, 23). In Fairley's report individuals with hookworm disease were non-reactive to the *Dirofilaria* antigen (8).

Using our stronger "B" antigen in 161 patients whose stools were examined, there was no significant difference in those with or without intestinal parasites. Forty-seven patients with positive stools had 93.6% positive skin reactions, and 114 patients with negative stools had 91.2% positive skin tests. On individuals with intestinal parasites who had never been in the Pacific Islands the skin reaction was always negative, but the number available was small, approximately 10. In 104 patients with negative stools, and again using the "B" antigen, the skin test was positive, indicating that the reaction was not produced by intestinal worms present at the time. This is strong evidence that the positive reactions obtained with *Dirofilaria immitis* antigen are not caused by other helminths, but the possibility in all instances has not been entirely eliminated.

quiescent. 263 of the 268 patients were evacuated to the U. S. A. This was thought wise because of the prevalent opinion that repeated reinfection might produce the more serious manifestations of filariasis, and because of the observation that residence in a temperate climate remarkably reduces the attacks of lymphangitis, which may recur soon after return to the tropics (22).

Sulfonamide therapy has been reported to be effective in treatment of the acute lymphangitis (1, 4, 7). However, microfilariae have been noted to persist in the blood during treatment with sulfonamides (7, 12) so that it cannot be said to be specific in its action against the parasite. We used sulfathiazole in full therapeutic doses in three of our more severe cases with fever and there was no apparent effect. Four patients developed acute lymphangitis while receiving sulfathiazole or sulfadiazine for the treatment of gonorrhea. In the early filarial lymphangitis we have given our reasons for not thinking the inflammation is bacterial. These few therapeutic observations would support this view.

Mapharsen was given to 21 patients an average of three doses each, one week apart, the first dose being 0.04 gm. and the remaining doses 0.06 gm. intravenously. It so happened that a patient receiving Mapharsen for fever developed typical lymphangitis six hours after his first injection. This led us to give it to 20 more individuals with clinical filariasis. In 17 it had no immediate effect. In one case there was exacerbation of epididymitis 24 hours after the injection. In the remaining two, acute lymphangitis occurred 6 and 24 hours after administration. The significance of this is in doubt. It could be coincidental, or it could be due to the death or irritation of the adult filariae.

Prophylactically, mosquito control is the obvious ideal procedure. Personal protection must be practiced both day and night because of the daytime biting habits of the *Aedes triseriatus*, the chief vector in the Pacific Islands east of the New Hebrides group (17).

served. Many of the genital cases had some thickening or nodularity of the spermatic cord or epididymis when last seen, but none of the lesions was very marked and they all tended to regress continuously. Since the common genital lesion is in reality a lymphangitis, it would seem unlikely that obstruction of the vas deferens would occur unless there were repeated attacks with resultant severe fibrosis. Other work has indicated this to be true (13, 18).

#### DISCUSSION

Of the many clinical manifestations of filariasis described extensively in the literature, we have seen only the earliest lesions. That these findings are due to infection with *Wuchereria bancrofti* is supported both by direct and indirect evidence. The direct evidence is contained in the paper by Wartman (26) on the pathology of the lesions which is based on the biopsies taken from seventeen of these patients. As is seen in his paper, the adult worms or an inflammatory reaction which is characteristic of their presence was found in nearly all of the biopsies. That the filariae do produce the pathological changes and clinical findings has been ably demonstrated by O'Connor (19, 20) and others and is supported by Wartman's findings.

All of the patients had opportunity to contract filariasis by living in known endemic areas. All but five of them had lived in Samoa or Wallis Island four months or longer. These five had been exposed only from two to three months. In addition, the men were living in close proximity to natives who are known to be reservoirs of the microfilariae. Our figures suggest a higher incidence of the disease on the second island mentioned before. However, variations of local conditions from island to island are unknown to us. Such factors as housing, proximity of native villages and mosquito-control measures must be evaluated by those on the spot. It is further pertinent that no patients having symptoms suggesting filariasis have yet been seen in this hospital who have not spent time in islands mentioned.

The clinical findings do not fit any other known disease except filarial lymphangitis and lymphadenitis. Our reasons for not thinking them to be caused by bacteria have already been given. In particular the centrifugal progress of the lymphangitis is not the rule in bacterial infection. The type of involvement of the genitalia seems characteristic. The very frequent involvement of the

spermatic cord, the less frequent inflammation of the scrotal skin and the absence of lower urinary tract infection are unlike other disease.

The frequency (53.3%) with which enlargement of the nodes in one or both epitrochlear regions was found indicate that this sign might be useful in a survey of troops who had lived for some months in an endemic area. Buxton found it to be a useful sign in his work in the Pacific and found it to be the physical sign most closely correlated with the presence of microfilariae in the blood (2). It has been stated by some (2, 6) that the lymphadenitis precedes the lymphangitis, but this has not always been true in our experience. 85.1% of the patients had some degree of palpable lymph node enlargement, but the remainder had only lymphangitis of extremity or genitalia. There were 20 cases who had a history of or were observed by us to have lymphangitis of an extremity in whom no adenopathy was found.

The rarity of severe constitutional symptoms deserves further emphasis. Fever was not the rule and most often quite mild. Malaise was rarely more than minimal and usually not present at all. Well-established cases in natives more frequently exhibit severe prostration and fever at the onset of the lymphangitis (22). The tendency toward recurrence of the symptoms over a period of months is again stressed.

The absence of microfilariae in the peripheral blood of this group deserves further comment. Lane (15) states that the earliest age at which microfilariae were found in the natives of the Pacific Islands was four years, and in other parts of the world fourteen months. This would suggest that there is a long period after infection before the larvae are produced or can reach the blood stream. The length of this period is unknown. If patients and troops from endemic areas could be observed for a long period of time some light might be thrown on this problem. Also the question of correlation of the presence of microfilariae in the blood with clinical symptoms of the disease, and the larger problem of the natural history of the disease after relatively short periods of exposure would be clarified.

The intradermal reactions impress us as being corroborative evidence of past or present infection with *W. bancrofti*. Care must always be taken in using controls along with the testing of the patients. There has been some difficulty in getting all lots of antigen to react uniformly, and all lots

used in this study were checked against non-infected individuals to eliminate unusual numbers of false positive reactions. Refinements in technique of preparation and purification of the antigenic principle of *D. immitis* await further work. Recently it has been found that to refilter our best ("B") antigen through a Seitz filter will remove some of its antigenic properties. This probably means that it is not a true solution and that perhaps part of the antigenic fraction is held in suspension in the saline solution. Ideally antigen prepared from either adult or larval forms of the homologous parasite should be used, but the adult form is most difficult to obtain because of its small size, and we have not had access to any reservoir of microfilariae. We did not make the diagnosis of filariasis on the basis of the skin test alone in any instance, and believe that it should be supported by clinical evidence. The test might be useful in a survey of a large number of individuals who have lived in an endemic area. Thus an approximation of the incidence of filarial infection could be obtained.

## SUMMARY

1. 268 cases of early filariasis are presented.
2. The infection was contracted in three Pacific islands. No patients were seen that had not been in these areas.
3. 263: (98%) of the patients had been in the endemic areas four months or longer.
4. The observed incubation period varied from eight to sixteen months when the period of observation ended, with history of attacks as early as three months.
5. The mode of onset and clinical findings are described in detail. The tendency toward recurrences, the lack of severe constitutional reactions, the characteristic lymphangitis of extremity or genitalia and the adenopathy particularly in the epitrochlear region are stressed.
6. The intradermal reaction with *Dirofilaria immitis* antigen was positive in 90.8% of patients and 10.5% of a comparable number of control individuals.
7. Elevation of the total number of eosinophiles in the blood was present in 64.3% of the cases.
8. It is suggested that enlargement of the epitrochlear nodes and the intradermal reaction might be used as survey methods in approximating the incidence of filarial infestation in personnel residing in endemic areas.

*N.B.*: We have been aware that work on this problem has been going on for some time in the localities affected and have had access to unpublished information gathered by R. W. Huntington, Jr. Since this paper has been written a preliminary report on filariasis in U. S. troops in the same region has been published by Dickson, Huntington and Eichold (U. S. Naval Med. Bull., 41: 1240-1251, Sept. 1943). Their findings, experiences with the skin test and conclusions closely correspond with our own. Their further work on the spot where the disease is endemic will be awaited with interest.

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# LESIONS OF THE LYMPHATIC SYSTEM IN EARLY FILARIASIS

WILLIAM B. WARTMAN<sup>1</sup>

From the Laboratory Service, General Hospital, A.P.O., San Francisco, California

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This report covers material removed at biopsy from soldiers with filariasis. The place of exposure to infection, the time spent in the endemic area, and the time of appearance of symptoms after removal to a non-endemic region are all well established.

## MATERIAL

This included 20 lymph nodes, and 4 cord-like structures from the lesions of acute lymphangitis, removed at biopsy from 17 soldiers, 18 to 26 years of age. They had not been exposed to filariasis prior to a 4 months residence in a certain island of the Southwest Pacific. One man (No. 771) had spent the entire time in Island No. 3.<sup>2</sup> The others were on Island No. 1 for 1 month, Island No. 2 for 2½ months and on Island No. 3 for 2 weeks. They were then in the Solomon Islands for 5 months before evacuation to a temperate region where filariasis does not occur. Filariasis and elephantiasis are infrequent in the Solomons and no cases of filariasis were observed in the many patients transferred to this hospital who had been stationed only there. However, the disease is highly endemic in the other islands numbered above. The men lived in primitive conditions on Island No. 2, but on Island No. 3 they lived on shipboard except for a few days shore leave. It is thus highly probable that most of the infections occurred on Island No. 2 and in 1 instance on Island No. 3.

Manifestations of the disease began 3 to 13 months after what appears to have been the first exposure. These were principally epididymitis, retrograde lymphangitis of the upper arms, together with enlargement of the epitrochlear, axillary and inguinal lymph nodes. Lasting a few days, these signs reappeared at irregular intervals of a few days, several weeks or even months.

<sup>1</sup>Lt. Col., Medical Corps, Army of the United States. On leave of absence from the Institute of Pathology, Western Reserve University and the University Hospitals, Cleveland, Ohio.

<sup>2</sup>For reasons of security, the names of the islands are not given. The numbers assigned correspond to those in the paper by Dr. Boyd King (4).

Upon discharge from this hospital for return to the United States, the men were in good health except for attacks of lymphangitis and epididymitis. None had developed elephantiasis, but they will be followed elsewhere.

## METHODS

Microfilariae were not found in thick or thin blood films nor by Knott's concentration method (5), in spite of examination at frequent intervals throughout the 24 hours of the day. Medical officers in the islands had had the same experience with these patients even though microfilariae were easily demonstrated in the blood of the natives of the same region.<sup>3</sup>

Blood counts from 13 patients showed 500 or more eosinophils per cmm., but 5 of these had concurrent helminthic infections. The Kahn test was negative in all patients.

Intradermal tests with 0.1 cc. of a 0.1% saline extract of *Dirofilaria immitis*, according to the technique of Fairley (2), were positive in all but 1 patient (No. 633). In the controls, only 10% were positive. The important clinical and laboratory observations are described by King (4) and are summarized in table 1.

The specimens consisted of 8 epitrochlear, 6 axillary, and 6 inguinal lymph nodes, as well as 3 cords from areas of acute lymphangitis in the epitrochlear region and 1 cord from a similar lesion in the axilla. Aerobic and anaerobic cultures from this material, using meat broth and blood agar streak and pour plates, incubated at 37°C. for 2 weeks, yielded no growth. Two lymph nodes were digested with gastric juice, but no worms were recovered.

The tissues, removed at different times of the day, were immediately fixed for from 15 to 24 hours in either 10% neutral formal-saline or Zenker-formol, embedded in paraffin and cut at 5 micra. Semi-serial sections were made in most of the

<sup>3</sup>Recently Lt. Col. G. G. Duncan has informed me that microfilariae have been observed in the blood of soldiers exposed to the disease, but free of any of its manifestations.



Abbreviations used in table: G.I., chronic granulomatous inflammation; R.E., reticulo-endothelial hyperplasia; E., tissue eosinophilia; N.H., hyperplasia of lymphatic nodules; A.L., acute lymphangitis; C.L., chronic lymphangitis; L.T., lymph thrombosis; T., thrombosis. Capsule = capsule of node and the subcapsular lymphatic sinus. Necator americanus were cultured from the hookworm ova in three stool specimens. Cultures not made in the other cases.

TABLE 1  
Summary of Clinical and Pathological Findings in 17 Cases of Early Filariasis

PATIENT	CLINICAL DIAGNOSIS	BLOOD EOSINOPHILES PER CMM.	STOOLS	autopsy	WORMS	LYMPH NODES, MICROSCOPIC FINDINGS	LYMPHATIC VESSELS, MICROSCOPIC FINDINGS
415	Acute lymphangitis, left arm. Acute left epitrochlear and left femoral lymphadenitis.	286	neg. x 1	10 Feb. 1913, 0840 hours. Left epitrochlear lymph node 1 x 0.5 x 0.7 cm. Pinkish grey & firm. Few fibrous adhesions on capsule.	2 gravid females, 1 male, probable more, all living, in pulp.	GI +++ worm capsule RE +++ worm E +++ NH +	AL +++ T Afferent vessel
453	Acute lymphangitis, left arm. Acute lymphadenitis, left epitrochlear region	721	Hookworm ova	25 Feb. 1913, 1215 hours. Epitrochlear lymph node. Firm, pale grey 1 cm. in diameter. 1 cm. of acutely inflamed lymphatic cord.	3 gravid females, 1 male. 1 female partly calcified. Male situated in lymphatic others at edge of tissue.	GI +++ diffuse RE +++ diffuse E +++	AL +++ T Necrotizing
460	Acute lymphangitis, left arm. Acute lymphadenitis, left axilla. Acute epididymitis and etiology undetermined.	1650	neg. x 1	26 Feb. 1913, 1030 hours. Left axillary lymphatic cord 2 x 1 x 1 cm. Firm & pinkish grey.	1 degenerating worm of unidentified sex in lymphatic. 1 gravid female at edge of tissue.	GI +++ RE +++ E +++	AL +++ RE +++ occlusive
512	Acute lymphangitis, right arm. Acute right epitrochlear axillary and inguinal lymphadenitis.	547	Hookworm ova	18 March 1913, 1630 hours. 2 right epitrochlear lymph nodes. One measures 1.1 x 0.6 cm., and the other 2.5 x 1.5 x 1 cm. Pale pinkish grey with intact capsules and slightly retracted moist cut surfaces.	1 adult presumably living, female at edge of section. Ova but no microfilaria in uterus. Foci of necrosis in capsule, presumably around dead worms.	GI +++ RE +++ NH +++ E +++	Normal
771	Acute epitrochlear lymphadenitis, bilateral.	498	Hookworm ova	31 July 1913, right epitrochlear lymph node 2.5 x 1.5 x 1 cm. Capsule intact, cut surface pinkish grey, moist and bulging.	6 degenerating worms some females. Marked fibrosis around worms.	GI +++ RE +++ E ++ NH +++	Normal
452	Acute lymphangitis, left arm. Acute left axillary and epitrochlear lymphadenitis.	703	neg. x 1	25 Feb. 1913, 1440 hours. Left epitrochlear lymphatic cord extending up arm. 1.5 x 0.3 cm. Firm and pinkish grey.	None	GI +++ RE +++ E +++	CL +++ occlusive
454	Acute lymphangitis left arm. Acute left epitrochlear lymphadenitis. Acute catarrhal jaundice. Etiology undetermined.	328	Hookworm & Trichocephalus trichiurus ova.	25 Feb. 1913, 1110 hours. Mass in left epitrochlear region 15 x 8 x 6 mm.	None	GI + RE 0 E 0	Normal

[illegible]

TABLE 1—Continued

PATIENT	CLINICAL DIAGNOSIS	BLOOD EOSINOPHILES PER CMM.	STOOLS	NOTES	WORMS	LYMPH NODES, MICROSCOPIC FINDINGS	LYMPHATIC VESSELS, MICROSCOPIC FINDINGS
569	Acute lymphangitis, left arm. Acute right epitrochlear and axillary lymphadenitis. Acute right epididymitis and funiculitis.	1206	neg. x 1	17 April 1943, 1030 hours. Left epitrochlear lymph node. Discrete 2 x 1 x 1 cm. Cut surface slightly retracted, smooth and pinkish grey.	None	GI ++ capsule RE ++ capsule E +++ diffuse NH +++	Normal
600	Acute lymphangitis, left arm. Acute epitrochlear lymphadenitis, bilateral	0	neg. x 1	30 April 1943. Left epitrochlear lymph node. Discrete 1.5 x 0.8 cm. At operation a small amount of thick creamy yellow fluid was expressed. Cultures negative. Node dissected and no sections made.	None	—	—
633	Acute lymphangitis, both arms, 6 weeks duration. Acute lymphadenitis, both epitrochlear regions. Generalized lymphadenopathy. Skin test negative.	1015	neg. x 1	25 May 1943. Left epitrochlear lymph node 3 x 1.5 x 1.5 cm. Capsule moderately thick, pale grey and opaque at one pole. Cut surface pinkish grey, moist and bulging.	None	GI ++ capsule RE ++ diffuse E + NH ++	Normal

specimens, but in some, complete serial sections were cut. Routinely the sections were stained with Harris' hematoxylin and aqueous eosin. When necessary the following were also used: Masson's trichrome stain, Verhoeff's elastica and van Gieson's connective tissue stains, Mallory's phosphotungstic acid hematoxylin stain, Foot's silver stain, Goodpasture's bacterial stain and Ziehl-Neelsen's stain for acid fast bacteria.

# RESULTS

The plan of presentation will be to discuss the morphology of the worms, the pathologic lesions

encountered in the lymph nodes and vessels, and wherever possible the histogenesis. This description is a composite of all the material, the details being shown in the photomicrographs and table 1.

## THE WORMS

Tissue sections do not permit fully satisfactory identification of these parasites, but photomicrographs showing certain features are included with this publication. According to the criteria of Yorke and Maplestone (11) the worms in the lymph nodes and vessels of these biopsies should be classified as phylum Nematelminthes; class Nematoda; superfamily Filarioidea; family Filariidae; subfamily Filariinae; genus *Wuchereria* (figs. 1 to 4). The worms are elongate-cylindrical in shape with a smooth, non-nucleated cuticula and a schizoele in which all the viscera are suspended. They are filiform worms, the females being approximately twice the size of the males and estimated to measure about 80 by 0.25 mm. Although tapering towards both ends, the terminations are bluntly rounded. They possess a gut but are without a proboscis; the mouth is short and lined with cuticula; the esophagus moderately long and muscular; and the midgut lined by a single layer of columnar cells. The posterior end, and the excretory and nervous systems could not be satisfactorily studied. With the exception of the vulva, the entire female genital system including the ovaries and double uterus, could be identified, whereas in the male worms only the testes could be recognized. The live female worms all contain large numbers of ensheathed microfilariae. Because of the continuity of the genital tract, the development of the microfilariae from the germinal cells of the ovary to the more mature forms in the distal part of the uterus can readily be traced (figs. 3, 4). The presence of the ensheathed larvae

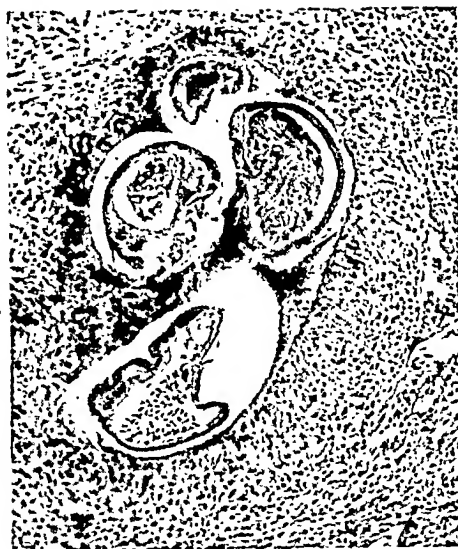


FIG. 1 (Biopsy 415). Gravid, female, filarial worms in an epitrochlear lymph node. Note the multitude of intrauterine microfilariae and the characteristic granulomatous tissue reaction around the worms. M.T.S.  $\times 200$ .\*

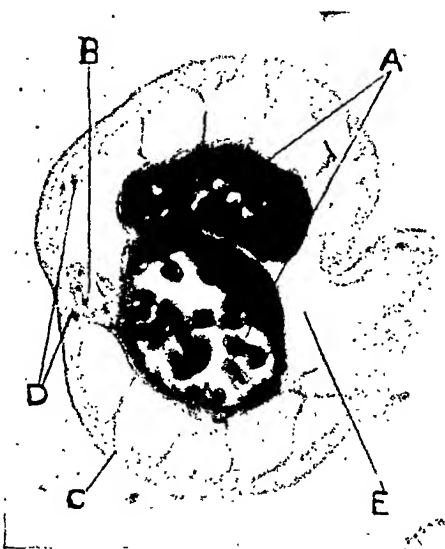


FIG. 2 (Biopsy 415). Cross section through the mid-portion of filarial worm showing "A" the double uterus with ensheathed microfilariae, "B" the intestine, "C" the cuticula, "D" two muscle cells, and "E" the schizoele. H.&E.  $\times 400$ .

\* The following abbreviations have been used throughout the legends in this paper: H.&E., Harris' hematoxylin and aqueous eosin; P.T.A.H., Mallory's phosphotungstic acid hematoxylin; M.T.S., Masson's trichrome stain; F.S.S., Foot's silver stain; V.G.V.S., Van Gieson's connective tissue and Verhoeff's elastica stains.

identifies the worms in the superfamily Filarioidea. Since the only other ensheathed microfilaria which



FIG. 3 (Biopsy 415). Tangential section through the upper portion of a female worm showing ovary with developing eggs. P.T.A.H.  $\times 400$ .

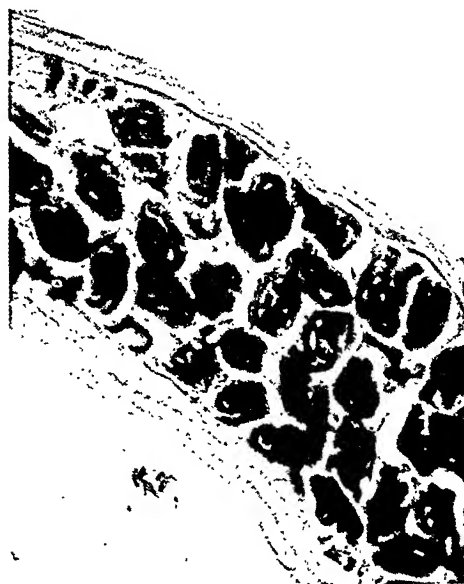


FIG. 4 (Biopsy 453). Immature larvae coiled within their sheaths in the mid-portion of the uterus. H.&E.  $\times 400$ .

is pathogenic for man, namely *Loa loa*, does not occur in the regions where these patients were

exposed, it is safe to assume that they belong to the genus *Wuchereria*. That they are *W. bancrofti* (nonperiodic form) is highly probable because that form is endemic in the 3 islands mentioned and is the species found in the blood of the infected natives of Island No. 2. The organisms were differentiated from *Microfilaria malayi* by the absence of terminal nuclei and the extension of the nuclei to within a short distance of the head end.

Determination of the number and sex distribution of the worms in the tissues could not be exact, because even in serial sections the tangle of worms was too intricate for certain differentiation. In

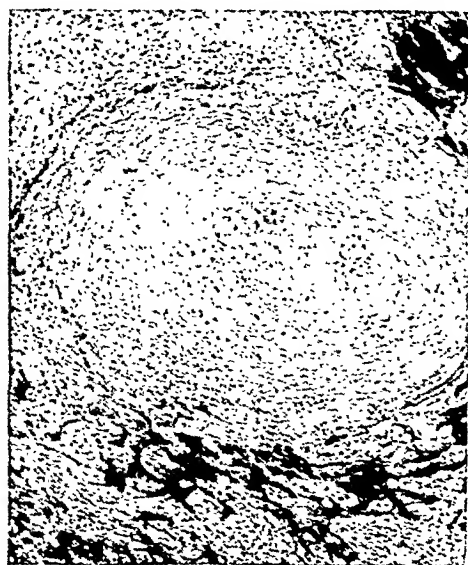


FIG. 5 (Biopsy 771). Small nodule (granuloma) around a degenerating worm in the cortex of a lymph node. Concentric layers of collagenous tissue have been laid down with proliferation of macrophages and exudation of lymphocytes and eosinophils at the periphery. Numerous bundles of hyperplastic and hyperchromatic fibroglial fibrils and collagenous fibers are present. P.T.A.H.  $\times 200$ .

general only a few worms were found, the largest number (in node 771) being approximately 6. This is in contrast to the large number reported by O'Connor and Hulse (8).

The encapsulation, degeneration and resorption of the worms are described below (fig. 5). In 1 instance the worm was calcified (fig. 6). Only occasionally did rapid death of the worm occur, leaving a small focus of necrotic material and cellular debris.

In these cases, microfilariae were not found in

the circulating blood, free in the tissue sections, nor in imprint smears from nearly all the specimens.

#### MICROFILARIAE IN THE BLOOD OF NATIVES OF ISLAND NO. 2

Six thin blood films from infected natives of this island, originally stained by Wright's stain, were decolorized and stained with Harris' hematoxylin. These were compared with type specimens from patients in Australia. Examination of all observable details indicates plainly that the parasites in the natives are characteristic of *Microfilaria bancrofti*. These were probably of the non-periodic form, since that form is endemic in these islands.



FIG. 6 (Biopsy 453). Partial calcification of a filarial worm in a lymphatic cord from the upper arm. H. & E.  $\times 200$ .

Thus it is reasonable to assume that the patients, like the native islanders, were infected by *Wuchereria bancrofti*.

#### MACROSCOPIC APPEARANCE OF THE LYMPH NODES

The lymph nodes were discrete, moderately enlarged and had thin intact capsules and firm, slightly bulging, moist, pinkish-gray cut surfaces. Occasionally filamentous fibrous adhesions had formed between the capsule and the surrounding fat.

#### TISSUE REACTIONS IN THE LYMPH NODES

For purposes of discussion the lymph nodes are divided into two groups, which will be described

separately; those containing adult filarial worms, and those in which, although there were no worms, nevertheless noteworthy tissue changes had occurred. In three of the biopsies the worms were alive, as far as could be determined, at the time of operation, whereas in the other two they were obviously dead. However, the tissue reactions were essentially the same whether the worms were alive or dead, and no evidence was obtained to indicate that, as is often stated, only degenerating or dead worms cause reaction in the tissues.

Three characteristic tissue reactions occur in lymph nodes in response to the presence of adult filarial worms: (1) granulomatous inflammation, (2) proliferation of the macrophage (reticulo-endothelial) system, and (3) tissue eosinophilia. As a matter of convenience these will be described separately but all three reactions occur together, and although one of them may be more conspicuous in some nodes than in others, careful search will often show all three. Collectively they form a pathologic picture which characterizes filariasis.

The terminology of Maximow and Bloom (6) for the cells of the macrophage or reticulo-endothelial system will be used throughout this paper.

#### THE GRANULOMATOUS INFLAMMATION

The granulomatous inflammation was found around the worms, in the lymphatic sinuses and in the capsule. The worms lay in enormously dilated cortical or medullary sinuses, and were surrounded by a wide zone of macrophages and reticular cells at the margins of which there were variable numbers of eosinophils, lymphocytes and foreign-body type giant cells (fig. 1). Necrosis was slight and involved only the hyperplastic cells lying within the internal reticular lamina of the affected lymphatic (fig. 7). Small numbers of fibroblasts were present, but there was no collagenous connective tissue, and the lesions were almost entirely avascular. An apparently significant feature was proliferation of argentophilic reticulum which formed an extensive network of delicate interlacing fibrils with occasional coarse fibers (figs. 7, 11).

The granulomas in the sub-capsular lymph sinuses and the capsule were of the same general pattern as those about the worms, but there were some minor differences (fig. 8). Necrosis was not observed and there was usually a considerable amount of mature collagenous connective tissue resulting in great thickening of these structures. The inflammation extended a short distance into



FIG. 7 (Biopsy 415). Marked proliferation of reticulum in the granulomatous inflammation. The inner reticular lamina of the affected lymphatic vessel, indicated by arrows, shows extensive disruption. F.S.S.  $\times 200$ .

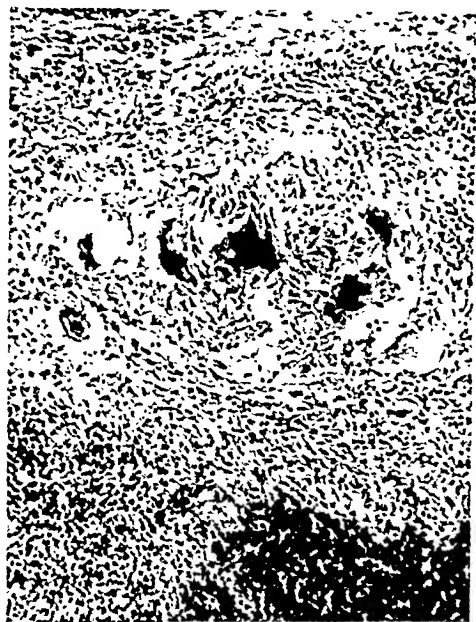


FIG. 8 (Biopsy 460). Granulomatous inflammation in the sub-capsular lymph sinus with nodule formation, numerous giant cells, large numbers of eosinophiles, and mature collagenous connective tissue. H.&E.  $\times 200$ .

the adjacent fat tissue, but spread only a little way along the trabeculae, sparing the cortical follicles, the hilum and the efferent lymphatics.



FIG. 9. (Biopsy 462). Early lesion in the capsule of a lymph node showing exudation of eosinophiles around a small vein. H.&E.  $\times 600$ .



FIG. 10 (Biopsy 415). Marked proliferation of macrophage cells in the vicinity of a living adult female filarial worm. The worm lies above the zone of necrosis. M.T.S.  $\times 600$ .

Focal condensation and increase in size and number of fibroglial and collagenous fibrils was constant, both around the worms (fig. 5) and in

inflamed areas in the cortical lymph sinuses, and resulted in bundle-like collections of deeply staining hyperplastic fibrils.

An early stage of the lesions, consisting of perivascular exudation of eosinophils and lymphocytes, was encountered in the capsule (fig. 9). This usually occurred about the veins or perivascular lymphatics and only later involved the adjacent artery.

The changes in the cortical lymphatic nodules were variable. Frequently the nodules were increased in number and size and there were foci of rapidly proliferating lymphocytes, although in

the worms where the cells formed a wide encircling zone with their long axes parallel in palisade fashion (figs. 1, 10). The cells farthest from the worms were well preserved whereas those immediately adjacent to them were necrotic. In between these two extremes the cells showed varying degrees of cloudy swelling and fatty degeneration and nuclear irregularities. The individual cells were large with abundant, faintly stained cytoplasm, which was frequently poorly defined and tended to blend with that of adjacent cells, and large round or oval nuclei having most of the chromatin condensed along the nuclear membrane. Small amounts of intercellular ground substance giving the staining reactions of collagen and a few fibrils were present. Some of the cells contained phagocytosed debris.

The reticular hyperplasia was most marked in two places, namely about the worms, and in the sub-capsular lymph sinuses, and was best shown by silver methods. In the former situation the fibers usually ran at right angles to the circular reticular layer of the involved lymphatic vessel, forming an intricate meshwork by the advanced degree of branching (figs. 7, 11). Both coarse and fine fibers were present, and although the former were isolated, the latter were intimately related to the hyperplastic cells, and in most instances arose from them.

In the sub-capsular lymphatic sinuses the tissue reaction was predominantly reticular. The reticulum formed an extremely dense network of intertwined coarse and fine fibers without any particular pattern, resulting in great thickening of the sinus and capsule. A relation to collagenous connective tissue was indicated by the fact that the fibers stained equally well with both silver and fuchsin.

A characteristic feature of the reticulo-endothelial hyperplasia was the presence of moderate numbers of foreign body type giant cells, which were presumably formed largely by fusion of cells and to a lesser extent by nuclear division without cell division (fig. 12). The margins were indistinct, often showing pseudopods, and the cytoplasm was abundant and moderately acidophilic. Numerous nuclei were distributed in the cell, but without any distinctive arrangement. Scattered fragments of chromatin and other debris were phagocytosed. At the edge of many of the cells there was a focal increase and condensation of fibroglial fibrils.



FIG. 11 (Biopsy 415). Proliferation of argentophilic reticulum about an adult worm showing the silver staining fibrils arising from the cells. F.S.S.  $\times 600$ .

some, and often in the same nodule, there were also reaction centers. These foci of lymphocytopoietic activity in the lymphatic nodules resulted in mechanical pushing aside of the surrounding reticular fibers so that the pattern varied. Each of the three types of lymphatic tissue—diffuse, loose and nodular—was present in variable amounts and transitions from one to the other were easily traced.

#### THE RESPONSE OF THE MACROPHAGE (RETICULO-ENDOTHELIAL) SYSTEM

This consisted primarily of proliferation of macrophages and argentophilic reticulum. The hyperplasia of macrophages was most striking around



## TISSUE EOSINOPHILIA

This was demonstrated best in material fixed in formol-saline and stained with hematoxylin and eosin. Large numbers of eosinophils were present in the granulation tissue about the adult worms, and in the sub-capsular sinuses and lymphatic cords, and there were small numbers in the lymphatic nodules and the hilum. Imprint smears of the fresh material always showed many eosinophils. Occasionally focal collections of them formed small eosinophilic "abscesses" in the tissues giving rise to a characteristic lesion.

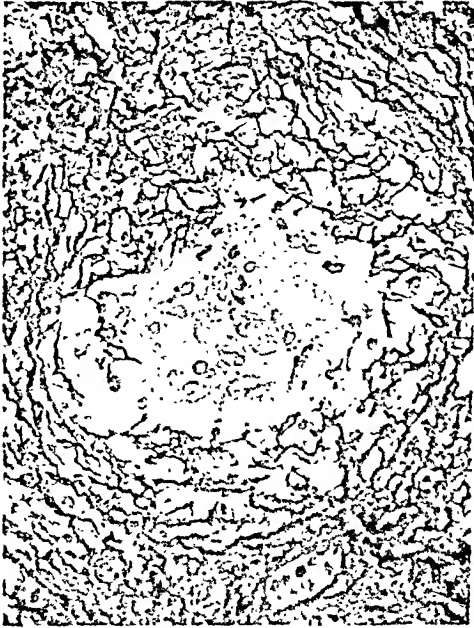


FIG. 12 (Biopsy 512). Typical giant cell exhibiting phagocytosis and the surrounding reticulum. F.S.S.  $\times 200$ .

The majority of the eosinophils were polymorphonuclear with a scattering of mononuclear forms. However, in the connective tissue of the capsule, and the adjacent fat, there were appreciable numbers of spindle-shaped cells with vaguely defined cytoplasm packed with large, strongly eosinophilic granules. Deeply stained, elongated nuclei with blunt ends were situated near one pole. Such cells evidently arose from the fixed tissues in the neighborhood, and their presence indicates that some of the eosinophils were of histocytic rather than hemocytic origin.

## LYMPH NODES IN WHICH NO WORMS WERE FOUND

In most of the specimens no worms were demonstrated. Often, however, these nodes were en-

larged and showed microscopic lesions, chiefly in the cortical sinuses and the capsule, similar to those in the worm-infested nodes, including granulomatous inflammation, great reticulo-endothelial hyperplasia and tissue eosinophilia. The lymph nodules were markedly hyperplastic and the pattern of reticulum was altered. Occasionally the infiltration of eosinophils was even more conspicuous than in nodes containing worms. The question naturally arises as to whether these alterations in nodes not containing worms are sufficiently characteristic to justify a positive diagnosis of filariasis. Such changes are rarely, if at all, encountered in regions where filariasis does not occur. When fully developed, the lesion gives strong presumptive evidence and, when correlated with satisfactory clinical manifestations, can be looked upon as confirming the clinical diagnosis. It is to be noted that they were not present in two of the specimens and that in one there was only follicular hyperplasia. Thus the disease does not necessarily excite reactions in lymph nodes, or may cause lesions not distinguishable from other forms of hyperplasia. There are intermediate degrees of alteration that are at best only suggestive.

## LYMPHATIC VESSELS

These disclosed a great variety of lesions, including hyperplasia of lining endothelium and of reticular cells in the walls, acute lymphangitis either with or without thrombosis, and fibrous obliteration. Occasionally simple lymph thrombosis occurred in vessels which otherwise appeared normal.

A common early lesion was swelling and hyperplasia of the reticulo-endothelium of the lymph vessels accompanied by perivascular exudation of eosinophils (fig. 13). As this hyperplasia was frequently focal it usually resulted either in nodular thickening of the wall (fig. 14) or in villous processes which projected into the lumen (fig. 15).

One way in which obstruction may develop in a lymph vessel was well illustrated in the specimens from two patients with retrograde lymphangitis. One of them (Case 453) showed the early stages in which the affected vessel was greatly dilated and the entire thickness of the wall and the surrounding fibro-adipose tissue was acutely inflamed. A living adult worm, presumably a male, lay within the lumen with some lymph and a moderate number of lymphocytes, and along the wall at one place there was mural thrombosis (fig. 16). This thrombus, which was composed of lymphocytes and

coagulated lymph, increased in size by accumulation of more lymph, and other cells until the lumen

little resemblance to a lymphatic vessel (fig. 18). It is this latter lesion which is presumably the

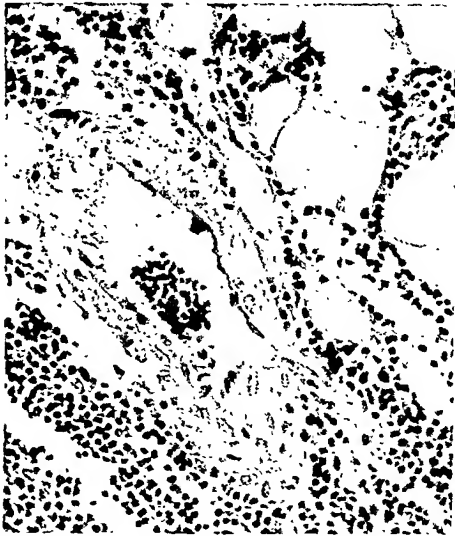


FIG. 13 (Biopsy 415). Early changes in a small afferent lymphatic vessel showing focal hyperplasia of the reticulo-endothelial cells of the wall, and perivascular exudation of eosinophiles. H.&E.  $\times 250$ .



FIG. 15 (Biopsy 415). Endothelial hyperplasia with villus formation in the lymphatic sinus of a lymph node. P.T.A.H.  $\times 250$ .



FIG. 14 (Biopsy 415). More advanced hyperplasia resulting in the formation of a projecting nodule. P.T.A.H.  $\times 250$ .



FIG. 16 (Biopsy 453). Figures 16, 17 and 18 illustrate the development of lymphatic obstruction and cord formation. Figure 16 shows an acutely inflamed and dilated lymphatic containing an adult worm. A beginning thrombus, indicated by the arrow, composed of coagulated lymph, lymphocytes and eosinophiles is attached to the wall. H.&E.  $\times 150$ .

of the vessel was occluded (fig. 17). The other patient (Case 452) showed the final stage where organization and fibrosis of the thrombus had ensued, leaving a dense cord-like structure bearing

basis for the development of lymph blockage and elephantiasis.

## BACTERIOLOGIC INVESTIGATIONS

There has long been a controversy as to whether the lymphangitis associated with filariasis is caused by filaria or by bacteria. This subject has been ably reviewed by O'Connor (7), who has established beyond reasonable doubt that the presence of bacteria is not necessary for the occurrence of most of the pathological changes and of all the inflammatory attacks associated with filariasis. The present study affords additional evidence that the lymphangitis and lymphadenitis are caused by filarial worms or some product elaborated by them. First, a careful and complete bacteriological examination of the specimens was made at the time of

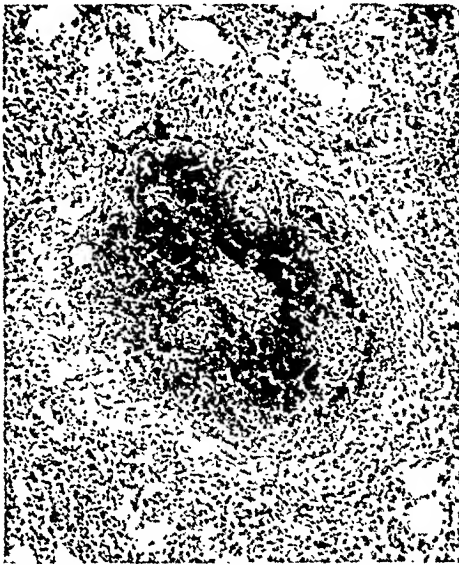


FIG. 17 (Biopsy 453). The lumen of the vessel is now occluded by the thrombus. H.&E.  $\times 150$ .

operation and later the tissues were stained for bacteria, but bacteria were never found. Second, in serial sections of the tissues it was possible to trace the histogenesis of the lesions from the early to the advanced stages and to show the development of lymphatic obstruction in the presence of the worms. Third, the lymphangitis was characteristically retrograde and not ascending as would be expected if it were of bacterial origin. Such observations clearly indicate that bacteria are not necessary for the development of the acute phases of filarial lymphangitis, although bacteria may, of course, secondarily invade already established lesions.

## DISCUSSION

From the foregoing description it is apparent that in the lymphatic system adult filarial worms incite an inflammatory reaction with the usual cellular phenomena. Regardless of the causative agent, the cellular reaction in the fixed tissues of the body in inflammation is essentially the same, consisting in the main of three phases; namely, (1) exudation of fluid and free blood cells or hemocytes, (2) stimulation of the macrophage or reticulo-endothelial system, and (3) reparative proliferation of fibroblasts and cicatrization. The vascular phenomena of inflammation, which occur chiefly in the early stages, were not a conspicuous feature

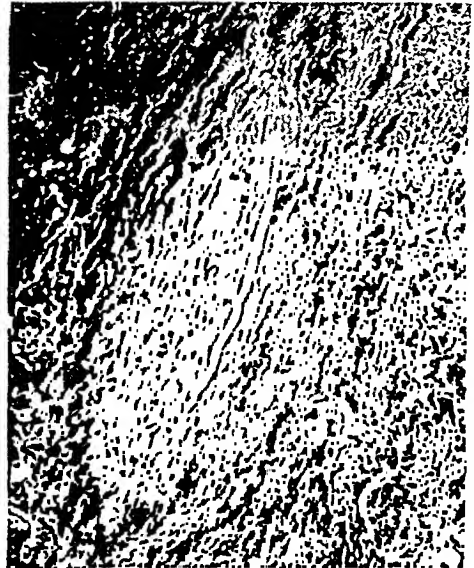


FIG. 18 (Biopsy 453). The end stage is represented by a dense cord of fibrous tissue bearing little resemblance to a lymph vessel. V.G.V.S.  $\times 150$ .

in the material under discussion. Variation in the inflammatory response may occur in different diseases depending upon the anatomical location of the lesions and the predominance of one or the other phase of the cellular reaction. Filariasis of the lymphatic system follows this general principle.

The hemocytic reaction, which is probably the first phase of the inflammation, was manifested chiefly by the blood-eosinophilia and exudation of eosinophils in the affected tissues. Other cells of exudation were also encountered, but in much smaller numbers. In the lymphatics this stage was sometimes accompanied by acute necrosis of



tients, because of the vicissitudes of war, lived under the same primitive conditions as the natives, whereas during peace times white people living in the same regions are usually quartered in the coastal settlements where anti-mosquito measures are in force.

As is well known it has been extremely difficult to obtain accurate information concerning the incubation period of filariasis, and the prevailing opinion has been that, "the incubation period lasts about one year (i.e. from the entry of the third stage infective larvae into the skin until microfilariae first appear in the peripheral blood), during which time there are no known symptoms" (1). Study of the patients reported herewith shows plainly that the acute stages of filariasis of the lymphatic system may occur as early as three months after exposure, not necessarily accompanied by demonstrable microfilariae in the peripheral blood.

*Note:* An abstract of this paper has been published in the Bulletin of the U. S. Army Medical Department (13). Since the preparation of this manuscript, the papers by Michael (14) and Flynn (15) have appeared describing a similar group of patients from the same area. Hartz (12) has published a paper demonstrating that lesions of the same character appear in filariasis as it occurs in the Netherlands West Indies.

Grateful acknowledgement is made to Professor Peter MacCallum for the use of the photomicrographic equipment.

#### SUMMARY

1. This is a report on the early lesions of acute filarial lymphadenitis and lymphangitis, due presumably to *Wuchereria bancrofti*.

2. Material was obtained by diagnostic biopsy on 17 otherwise healthy young white men who had resided in the islands for 4 months and were exposed to the bites of mosquitoes harboring the non-periodic form of *Wuchereria bancrofti*.

3. The clinical manifestations included acute epididymitis, acute transient retrograde lymphangitis and lymphadenopathy especially of the upper extremities. Intradermal tests were positive in all but one case. Microfilariae were not demonstrated in the peripheral blood during their stay in the hospital and elephantiasis did not occur.

4. Adult male and female filarial worms were found in five of the specimens. Both living and

dead worms were present and the females contained in their uteri large numbers of eggs and microfilariae which appeared morphologically mature. No free microfilariae were found in the tissues.

5. Cultures of the biopsies, as well as tissue sections specially stained for bacteria, were negative, indicating that the lesions were due to the worms and not to bacteria.

6. The tissue reactions in the nodes consisted of granulomatous inflammation with marked hyperplasia of the macrophage (reticulo-endothelial) system and tissue eosinophilia. The lymphatic vessels showed reticulo-endothelial hyperplasia, lymph thrombi, and varying degrees of inflammation with or without thrombosis.

7. It is suggested that the absence of microfilariae from the blood may be due to the avascular nature of the granulomas, the hyperplasia of macrophages, and the small numbers of worms found in the specimens.

8. The history of these patients proves that white persons can be infected during short visits to endemic areas, and that signs and symptoms of filariasis may develop as early as 3 months after the first exposure to infected mosquitoes.

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any symptoms suggestive of clinical filariasis. These were tested with the antigen made from microfilariae of *W. bancrofti* and also with the dried pulverized leukocytes. In every case there were negative reactions to both test antigens.

#### CONCLUSIONS

A test antigen has been prepared from the dried pulverized microfilariae of *Wuchereria bancrofti*. This material gave a positive precipitin reaction in 2 of 26 serums from patients with circulating microfilariae but without clinical symptoms and 3 positive tests from 14 patients with clinical filariasis but negative for parasites in the blood.

In 10 control serums tested with the antigen from microfilariae all the tests were negative. No positive reactions were observed in any of the serums tested with the dried pulverized leukocytes.

#### Acknowledgments

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# DEATH DUE TO ESTIVO-AUTUMNAL MALARIA

## A RESUMÉ OF ONE HUNDRED AUTOPSY CASES, 1925-1942

B. H. KEAN<sup>1</sup> AND JOHN A. SMITH<sup>2</sup>

*From the Board of Health Laboratory, Gorgas Hospital, Ancon, Canal Zone*

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Between January, 1925 and June, 1942, autopsies were performed at the Board of Health Laboratory, Gorgas Hospital, Ancon, Canal Zone, on 100 patients who had died of estivo-autumnal malaria. The purpose of this paper is to provide a resume of those 100 cases.

A total of 6,214 autopsies was performed during the period under consideration, so that the autopsy percentage on cases of malaria was 1.6 per cent. The autopsy percentage of the Board of Health Laboratory for all deaths was 60 per cent. Sixty-six patients came from Gorgas Hospital; the clinical data on these were fairly complete. The remaining 34 patients died at Colon Hospital, Corozal Hospital, Army post hospitals, or were coroner's cases. The clinical data on many of these patients were inadequate.

### SEASONAL DISTRIBUTION

Deaths occurred in all months. Two poorly defined peaks were present: in May and June, and in December and January. These peaks could be correlated with the general morbidity of malaria on the Isthmus of Panama. The number of autopsies each year varied from 2 to 10; no trend was recognizable.

### SEX, AGE, AND RACE<sup>3</sup>

Seventy patients were males and 30 were females (see table 1). Among the United States citizens there were 10 males to 1 female; among the West

Indians were 2 males to 1 female; among the Panamanians the sex ratios were equal. These figures reflect the sex distribution of the races on the Isthmus. The average age of the 100 patients was 22.6 years. The average age of the males was 24.6 years, with a range from 2 months to 66 years. The average age of the females was 13.9 years, with the range from 8 months to 53 years. Although there were five times as many West Indians as Panamanians in the Gorgas Hospital population, there were more than three times as many deaths from malaria amongst Panamanians as West Indians. The explanation is simple. Most of the West Indians lived in the sanitized Canal Zone,

TABLE 1

*Race and sex distribution (figures in parentheses indicate average age of race)*

	MALE	FEMALE	TOTAL
	yrs.	yrs.	
Panamanians.....	19 ( 8.6)	20 ( 5.2)	39
United States citizens..	30 (35)	3 (43)	33
British West Indians..	8 (17.9)	4 (37)	12
All others.....	13 (36.9)	3 (13)	16
Total.....	70	30	100

whereas many of the Panamanians in this series lived under more primitive conditions. Practically all of the United States citizens were soldiers who acquired their infection on maneuvers or on small outposts.

Two thirds of the females were Panamanians whose average age was 5.2 years. Of the 39 Panamanians who died, 34 were 10 years or younger. These figures confirm the experiences of others. Strong (1) has written: "The native children, to a striking degree, harbor parasites and to them in many tropical localities malaria is a prime cause of death." If the Panamanian survives his childhood attacks, apparently, he is not likely to die of malaria.

<sup>1</sup>Pathologist, Board of Health Laboratory. Captain, M.C. A.U.S.

<sup>2</sup>Staff Physician, Gorgas Hospital.

<sup>3</sup>In this paper the term race is used colloquially, meaning a group of people having similar appearance, customs, and language. The United States citizens are either white, employees of the Panama Canal or military personnel. The Panamanians are the native Latin Americans, many being mestizos. The British West Indians are Negroes born in Jamaica, Barbados, and other West Indian islands, who were imported to the Isthmus during the construction period of the Panama Canal, and their descendants.

## PREVIOUS ATTACKS OF MALARIA

Of the 100 patients who died of estivo-autumnal malaria, 29 had had no previous attacks, 24 had had 1 previous attack, 3 had had 2 previous attacks, 3 had had 3 or more previous attacks, and in 41 instances data on this score were not obtained.

*Duration of Symptoms*

The average duration of symptoms (see table 2) before hospital admission in these fatal cases was 4.3 days with the range varying from four and one-half hours to twenty-one days. Twenty-three patients had symptoms of not more than one day's duration before hospitalization, and yet they died. Many of the patients came from a class which does not complain until sick enough to die. Of these 23, 13 were admitted to Gorgas Hospital and 10 to other hospitals and dispensaries.<sup>4</sup>

TABLE 2

*Duration of Symptoms Before Admission*

Less than 1 day.....	8
1 day.....	15
2 days.....	6
3 days.....	12
4 days.....	8
5 days.....	4
6 days.....	6
7 days.....	8
8-21 days.....	11
Unknown.....	22
Total.....	100

*Type of Temperature*

No particular conclusions could be drawn from a study of the temperature charts. Some patients had high fever; others had little elevation of temperature.

*Clinical Signs and Symptoms*

*Vomiting* was present in 53 patients, absent in 19, and not mentioned in 28. *Headache* was present in 29, absent in 39, and not mentioned in 32. *Chills* were present in 34, absent in 35, and not

<sup>4</sup>Appreciation of these facts led to the institution of energetic measures for the early diagnosis of malaria by the thick film method and the immediate institution of anti-malaria therapy. This has resulted in a distinct drop in the mortality rate at Gorgas Hospital during the past two years.

mentioned in 31. The other signs and symptoms, as recorded by the clinicians, may be divided into two main categories: cerebral and cardiovascular.

*Cerebral*.—Coma or stupor was noted in 31, convulsions in 22, restlessness in 19, irrationality in 17, twitching in 11, loss of sphincter control in 11, delirium in 10, lethargy in 10, altered reflexes in 9, hiccough in 6, violence in 5, miosis in 3, spasticity of extremities in 4, meningismus in 3, toxic psychosis in 2, nystagmus in 1, and vertigo in 1.

*Cardiovascular*.—Fast, thready pulse was observed in 16, shock (mentioned by clinician) in 11, dyspnea in 10, cyanosis in 9, ascites or edema in 3, and gallop rhythm in 1.

*Miscellaneous Symptoms*.—Diarrhea was noted in 11, jaundice in 10, anuria in 6, gastro-intestinal bleeding in 6, "blackwater" in 5, backache in 3, dysphagia in 1, hematuria in 1, abdominal pain in 1.

*Liver*.—The liver was not palpable in 60 patients, palpable in 10, doubtfully palpable in 4, and information was not available in 26 instances.

*Spleen*.—The spleen was not palpable in 45, palpable in 21, doubtfully palpable in 4, and information was not available in 30 instances.

*Degree of Clinical Infection*

Of the 66 patients upon whom data were available, 30 had severe infections, 7 had moderate, and 29 had light infections. Unfortunately, no consistent definitions of these terms were employed. For practical purposes it was assumed that a severe infection was one in which more than 3 per cent of the erythrocytes were parasitized; a moderate infection was one in which 1 to 3 per cent of the erythrocytes were parasitized; less than 1 per cent parasitization was considered light.

That 29 out of 66 patients should die with light infections is noteworthy. It is possible, of course, that if serial blood films had been examined during the first twenty-four hours, some of the patients who were at first thought to have light infections would later have been shown to have moderate or heavy infections. And in some instances, the infection may have appeared light because the patient had resorted to the use of medication before professional advice was sought. Nevertheless, the clinician considered and treated many of these 66 patients *as though* they had light infec-

tions. While the number of parasites in the peripheral circulation may be the best available means of judging the seriousness of the illness, it is often deceptive. We have seen patients with 20 per cent infection of their erythrocytes who recovered and some with 0.5 per cent infection who died.

**Therapy.**—The therapy may be divided into categories depending upon the amount of drug the patient received each day, or would have received if survival had permitted the routine to be continued in those patients who died within the first twenty-four hours. In table 3 is recorded the dosage in relation to the degree of infection. Data were available in 62 patients.

Within the first twenty-four hours after admission, a total of 35 patients died: 17 with severe infections, 3 with moderate infections, and 15 with

TABLE 3

DEGREE OF INFECTION	DOSAGE				TOTAL
	Quinine (over 3 grams per day)	Quinine (between 2 and 3 grams per day)	Atabrine alone (over 0.5 gram per day)	Mixed treatment, quinine and atabrine	
Severe.....	19	1	4	4	28
Moderate....	2	1	1	3	7
Light.....	15	5	2	5	27
Total.....	36	7	7	12	62

light infections. Of the 36 patients receiving heavy quinine therapy (over 3 grams per day), 17 died within twelve hours after admission to the hospital; of these 17, 8 had light infections. Within the next twelve hours, 8 more died, of whom 4 had light infections. The death of 12 patients with light infections within twenty-four hours after admission, despite heavy quinine therapy, emphasizes the inadequacy of the apparent degree of peripheral infection as an index of the seriousness of the illness.

#### Autopsy Data

No attempt will be made to provide a summary of all the autopsy findings. Only some of the facts pertinent to the problem of malaria will be considered.

**Brain.**—Parasites were found in 71 patients, not found in 28, and in 1 case the record was

"doubtful." **Pigment** was recorded as present 68 times, absent 31 times, and questionable once. Plugging of the capillaries was studied. In 81 patients the statement was made that the capillaries were not plugged. In 16 the capillaries were "packed," "filled," "engorged," or "plugged with malaria parasites and pigment." In 2 instances the presence or absence of plugging was not recorded, but "many" estivo-autumnal parasites were noted in the brain. In 1 case the material was not satisfactory.

**Spleen.**—Parasites were present in 68, absent in 30, and examination was not made in 2. **Pigment** was present in 91, absent in 8, and examination was not done in 1.

**Blood.**—Parasites were present in 66, absent in 30, and in 4 instances no examination of the blood was made.

**Bone Marrow.**—Parasites were present in 58, absent in 29, and in 13 instances the marrow was not examined. **Pigment** was present in 64, absent in 23 cases, and in 13 instances the rib marrow was not examined.

**All Organs.**—In 98 out of the 100 cases, there were parasites or malaria pigment found in one or more organs. The remaining 2 patients had light infections; 1 died of blackwater fever and the other of chronic nephritis.

#### Type of Death

Although all of the 100 patients died of malaria, it was possible to subdivide the cases into several types of death based essentially upon the clinical course.

Shock.....	15
Doubtful shock.....	7
Cerebral malaria.....	12
Blackwater fever.....	5
Uremia.....	4
Ruptured spleen.....	3
Bronchopneumonia.....	3
Bloody diarrhea.....	1
Purpura.....	1
Other complications.....	3
Unclassified.....	46

#### Discussion of Type of Death

It is difficult to disagree with the recent statement of Rigdon (2) that the many clinical and pathological observations which have been made on malaria "have not been adequate to explain the

mechanism of death in acute cases of *Plasmodium falciparum* infection."

Seyfarth (3) presented a classification of the various forms of death in acute malaria:

- (1) Septicemic form (30 per cent of all deaths)
- (2) Cerebral form (55 per cent)
- (3) Cardiac or algid form (14 per cent)
- (4) Renal form (1 per cent)

Suprarenal form, pancreatic form, and rupture of the spleen were also recorded.

In our own material it was impossible to classify 46 per cent of the cases. Of these, 30 belonged to the group of 34 patients who did not die in Gorgas Hospital and upon whom clinical data were incomplete. Many of these represented the most severe type of malaria and it is possible that most would have been included by Seyfarth in his septicemic or cerebral forms. This left 16 Gorgas Hospital patients upon whom it was considered impossible to assign a particular type of death. Of the 54 instances in which classification was attempted, most fell into the shock and cerebral groups.

Shock as a factor in malaria death has been introduced with some trepidation. Although the voluminous literature on malaria contains numerous references to the "algid" form of death, the "cardiac" form, syncopal form, choleraic form, circulatory failure, cardiorespiratory failure, etc., definite mention of shock as a factor in malaria deaths has been rare. Dudgeon and Clarke (4) were impressed by the "observed frequency of cardiovascular phenomena." They stated: "It has been noticed during this epidemic of malaria in the Salonika Army that sudden death was not uncommon, and the committee which was appointed to inquire into and report on the fatal cases of malaria which occurred in the Salonika Army noted that a large proportion of the cases died within 24 hours, and no less than 57 per cent died within the first two days of admission to the hospital. Further, collapse and even sudden death occurred as an initial phenomenon of the disease in this epidemic."

In a recent article Rigdon (2), reporting the death of a 7 year old child, cautiously stated "the pathological lesions may have resulted from shock." Cannon (5) wrote "one feature of pernicious malaria which is noteworthy is the vascular injury, as revealed by generalized fatty degeneration, hemorrhages into the brain, purpura, etc. Such a condition should certainly predispose to loss of

fluid elements of the blood similar to that in shock, and as it does particularly in the algid forms of pernicious malaria." Elevated plasma potassium levels in man and monkeys infected with malaria have been reported by Zwemer, Sims, and Coggeshall (6), and in canaries by Velock and Scudder (7) who mentioned anaphalactic shock in relation to malaria.

In the early phases of our review of this material, the descriptions of the clinical symptoms as recorded in the doctors' records and in the nurses' notes pointed again and again to shock.

Two case reports may be presented as illustrative of the occurrence of shock in patients with malaria. Case I is taken from our series of 100 deaths. The patient in Case II was recently treated in Gorgas Hospital and recovered.

*Case I.* Mc. T., 1 37 year old white male, was admitted, acutely ill, having had symptoms of malaria for seven days. Examination of the blood showed heavy parasitization of the erythrocytes with *Plasmodium falciparum*. The patient was given 0.2 gram of atabrine by mouth three times at six-hour intervals, one dose of which may have been lost by vomiting, after which he received 0.1 gram of atabrine three times at eight-hour intervals. Forty-four hours after admission, the patient was given 2 grams of quinine by mouth, but he vomited shortly thereafter, so how much of the quinine was absorbed is not known. Forty-five hours following his admission, the patient suddenly and unexpectedly went into a state of extreme shock. The blood pressure could not be obtained in either arm, the radial pulse was imperceptible, the skin was cold and clammy. External heat was applied, and stimulants were administered, but the patient died three hours later. Two hours before death he had been given an additional 0.3 gram of atabrine intramuscularly.

A complete autopsy was performed sixteen hours after death. The nail beds were cyanotic. The leptomeninges were moderately congested and slightly edematous. The grey matter of the brain appeared injected and bright pink. The mucosa of the laryngo-epiglottic folds was shrunken as if there had been antemortem edema. There was a little frothy fluid in the trachea. The dependent portions of the lungs were somewhat congested. The liver was enlarged, weighing 2,300 grams; the edges were slightly rounded; the cut surface was of the "early nutmeg" type with pale grey-yellow parenchyma and congested sinusoids. The spleen was enlarged, weighing 500 grams; the capsule was smooth, glistening, and tense; the pulp was dark brownish-red; the trabeculae were prominent. The esophagus was slightly dilated. The stomach was distended and contained gas and about 100 cc. of

faintly blood-stained fluid; the mucosa of the fundus was markedly congested. The small and large intestines were moderately congested and edematous; there were no ulcerations or foci of gross hemorrhage.

In the histologic sections the leptomeninges were congested and the capillaries of the brain were engorged. Only an occasional estivo-autumnal ring form was seen in the erythrocytes. No "cerebral plugging" was noted. The vessels in the lungs were engorged. Red blood cells were present in the serous exudate which filled some of the alveoli. The heart muscle fibers were pale and swollen. The peritracheal nodes were congested and edematous. There was central engorgement in the liver and some cloudy swelling of the parenchyma. The spleen was congested. The capillaries of the stomach were filled with blood. All the internal organs contained parasitized erythrocytes and some malaria pigment within the capillaries. Twenty-five per cent of the erythrocytes within the peripheral blood were parasitized.

These changes were recorded objectively by a pathologist unconcerned with the problem of shock in malaria. If the criteria of Moon (8) are accepted, the patient had many of the pathologic changes seen in cases of shock. It is recognized that more intensive, early therapy might have prevented the death of this patient.

*Case II.* M. T., a 34 year old male Salvadorean, entered the hospital complaining of an abrupt onset of chills, fever, headache, backache, and general malaise three days prior to admission.

On arrival in the hospital he was tired and weak, but this condition was not considered serious. His temperature was 101 F. His pulse rate was 82 per minute and his respirations were 20 per minute. The blood pressure was 95 mm. of mercury systolic and 65 mm. diastolic. Except for a moderate injection of the throat and a barely palpable spleen, the physical examination was negative. The blood film showed moderate infection with *Plasmodium falciparum*. Two grams of quinine sulphate were administered by mouth thirty minutes after admission. Nine hours after admission the patient was found with a cold and clammy skin, an imperceptible pulse, a temperature of 95 F., and a blood pressure of 60 mm. of mercury systolic over 40 mm. diastolic. He was given shock treatment, consisting of elevation of his feet, application of external heat, an intravenous infusion of 1,000 cc. of saline with 5 per cent glucose, and an infusion of 250 cc. of plasma. After these measures the pulse improved in quality and the blood pressure rose to 86 mm. of mercury systolic and 56 mm. diastolic. Thirteen hours after admission the patient was again in good condition, and from that point his convalescence was uncomplicated. No abnormal reaction to quinine was noted. It was the impression of the attending physicians that the anti-shock measures were responsible for the patient's recovery.

It is a popular medical opinion that many patients who die of estivo-autumnal malaria have a cerebral type of death due to the plugging of the capillaries of the brain by thrombi or emboli of malaria parasites and pigment. Text book illustrations of plugging merely show a capillary of the brain filled with erythrocytes, most of which are parasitized. The pathologists, in the current series, obviously employed these conventional criteria of plugging and we have not modified their records.

Of the 56 patients classified as to type of death, 12 had a *clinical* course which properly might be included under the heading of "cerebral," although twice as many patients had a major cerebral symptom such as a convulsion during the course of the illness. In the autopsy protocols of 10 of these 12 patients were statements that the capillaries were not plugged. In the other 2 cases definite statement concerning plugging was not made, but in one protocol the pathologist recorded a "few E. A. rings" in the capillaries of the brain and in the other a "moderate number" of parasites were seen in the brain. In not a single one of these 12 cases were the pathologists sufficiently impressed by the histologic appearances to have employed the term "cerebral plugging."

Of the 16 cases in which plugging was mentioned, 11 belonged in the group of 34 in which clinical data as to the type of death were not available and who probably received less treatment than those who died in Gorgas Hospital. Of the remaining 5, 2 were listed under "shock" death, and 3 in other categories. None were listed under "cerebral" death.

Certainly no obvious correlation between cerebral malaria and cerebral plugging can be recognized in our material. It is of course possible that the treatment which the hospital patients received washed out the cerebral plugs before death. Whether this view is naive rationalization or has basis in fact awaits further investigations, possibly along the lines reported by Kniseley, Stratman-Thomas, and Elliott (9).

We wish to emphasize that the limitations of our method of study, based upon hospital charts and protocols of routine autopsies, should not be overlooked when considering the relationship between cerebral malaria and cerebral plugging. If the clinical data had been recorded in greater detail and if the autopsy material had been studied by special methods the lack of correlation might not have been so striking.

## SUMMARY

1. The records of 100 patients who died of estivo-autumnal malaria and upon whom autopsies were performed at the Board of Health Laboratory, Gorgas Hospital, Ancon, Canal Zone, between 1925 and 1942 were reviewed. One and six-tenths per cent of all autopsies (6,214) performed during that period were on patients who died of estivo-autumnal malaria.

2. Deaths occurred in all months, although questionable peaks in May-June and December-January were present.

3. Of 39 Panamanians in the series, 34 were children 10 years or younger.

4. The duration of symptoms before hospitalization varied from four and one-half hours to twenty-one days. Twenty-three patients had symptoms of not more than one day before hospitalization and yet they died.

5. The degree of parasitization of the peripheral blood was not a wholly adequate index of the seriousness of the illness since 12 patients with light infections died within twenty-four hours of admission despite heavy treatment with quinine.

6. Some of the classical signs and symptoms of malaria such as chills, headache, vomiting, palpable liver, and spleen were absent in one third to one half of the patients.

7. The clinical and pathologic changes which have been described in shock were recorded in one third of the patients upon whom classification of the type of death was possible.

8. No correlation between cerebral malaria as noted clinically and cerebral plugging as recorded at autopsy was apparent in our material.

9. Two case reports illustrating the occurrence of shock in patients with malaria were presented. Anti-shock measures were believed to have saved the life of one patient.

## Acknowledgment

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# ON THE PREPARATION AND PROPERTIES OF ANTIGENS FROM *PLASMODIUM KNOWLESI*<sup>1</sup>

ANNA DEAN DULANEY AND DEMPSIE B. MORRISON

*From the Division of Pathology and Bacteriology and the Department of Chemistry, University of Tennessee College of Medicine, Memphis*

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A saline extract of *Plasmodium knowlesi* parasites has proved to be a dependable antigen for use in complement fixation tests for malaria. The results of this test on 675 individuals have been reported (Dulaney, Stratman-Thomas, and Warr, 1942). A sensitivity of 81.6 per cent, as evaluated by thick blood film findings, was demonstrated. Reactions listed as nonspecific were obtained with 31 (8.7 per cent) of 358 sera from presumably nonmalarious individuals. However, the 31 positive sera included 14 specimens from patients known to have leprosy, Chagas' disease, or yellow fever, and all came from malarious areas.

A conservative attitude toward these and a large number of additional results of complement fixation in malaria would appear to justify the opinion that at the present time the test is more specific than sensitive. The increasing interest in the test and recognition of its diagnostic possibilities emphasize the need for a more sensitive antigen and for fundamental information regarding the nature of the antigenic substance in the malaria parasite.

The following types of parasite products, other than saline extracts, have been studied during the past year:

1. Phosphate buffer extracts of wet and dried parasites
2. Barbiturate buffer extracts of wet and dried parasites
3. Solutions of parasites obtained by treatment with barbiturate buffer and NaOH
4. NaOH solutions of parasites.

Parasites were obtained from laked red blood cells of infected monkeys. The course of the infection was followed and the blood drawn when the monkey showed a high percentage of red cells containing mature parasites. The method of har-

vesting and treatment of parasites has been described (Dulaney and Stratman-Thomas, 1940).

## METHODS

The water-bath method of incubation was employed for the complement fixation tests reported in 1942. For the past 2 years the Kolmer Wassermann technic (Kolmer, 1943) has been used with excellent results. It was adopted with the idea that laboratories performing this test routinely could easily perform complement fixation tests for malaria. Titration of complement and hemolysin are carried out in the usual manner. The test is done with one-fifth amounts<sup>2</sup> of all reagents in order to conserve antigen and to permit more tests with the same sample of serum. The serum is diluted 1:2½ by adding 0.45 cc. of saline to 0.3 cc. of serum. One-tenth cc. of the diluted serum is combined with 0.1 cc. of antigen and 0.2 cc. of complement representing ½ of the full 2 unit dose. Controls of all reagents are included. After overnight incubation in the refrigerator and 10 minutes at 37 C., 0.1 cc. of a 2 per cent suspension of sheep cells and 0.1 cc. hemolysin (carrying ½ of 2 units) are added, and readings made after further incubation for 30 minutes at 37°C. Kahn tubes have been used for these tests. Readings of negative or strongly positive reactions are easy. Intermediate reactions may be checked by centrifugation and by comparison with standards.

The dose of antigen has been determined by titration with known positive and negative sera. The antigen is diluted in serial fashion and 0.1 cc. amounts combined with 0.1 cc. of serum (1:2½) and 0.2 cc. of complement. The 0.1 cc. amount of the highest dilution of antigen giving a 4+ reaction with the positive serum is arbitrarily called the unit. Such a dilution must of course give no reactions with the negative serum or in the anti-complementary controls. The antigen dose for tests has consisted of 2-4 units.

<sup>1</sup>The studies on which this paper is based were made possible by support given by the Tennessee Valley Authority through the Division of Preventive Medicine of the University.

<sup>2</sup>This is the micro Kolmer test, with minor modifications (Kolmer, 1942).



## PREPARATION AND ACTIVITY OF ANTIGENS

## 1. Saline Extracts

The preparation of saline extracts as described previously (Dulaney and Stratman-Thomas, 1940) has not been altered. Dried parasites are ground with saline, frozen and thawed 4-6 times, and centrifuged. The supernate is distributed in ampoules and frozen. Small amounts of pigment which flocculate during refrigerator storage are removed by centrifugation before diluting the antigen for use in tests.

It has been found that one freezing and thawing releases into the extract only a part of the antigenic material. When the residue obtained by centrifugation is taken up in the original volume of saline (10 cc. per 0.1 gm. of dried parasites) and again frozen and thawed 4 times, the resulting supernate has one-fourth to one-half the antigenic activity of the first. Third and fourth extracts may be as active as the second.

The first extract is a deep amber color, clear and sparkling; the others almost colorless. With 4 successive extractions a calculated total of 1200-1600 antigenic units may be obtained from 0.1 gm. of dried parasites (table 1). With the average yield of 0.5 gm. of parasites per monkey it is evident that 6000-8000 units of antigen may be obtained from one animal. The parasite yield has reached 0.8-1.0 gm. in large and heavily parasitized monkeys.

## 2. Phosphate Buffer Extracts

Highly active antigens may be prepared by treating wet or dried parasites with M/10 phosphate buffer of pH 7.8-8.0. Freezing and thawing or extraction at room or refrigerator temperatures may be employed. Such preparations are 6-8 times more active than the saline extracts.

Since it is difficult to determine the weight of wet parasites the following comparison of antigenic activities was carried out with dried parasites from a pool of 6 monkeys.

Two 0.1 gram samples of dried parasites were each ground with 10 cc. of phosphate buffer and labeled (a) and (b). A third sample was ground with 10 cc. of 0.9 per cent NaCl (c). Preparation (a) was frozen and thawed 4 times, centrifuged, and the clear brown supernate removed and designated P-FT-1. The residue was mixed with 10 cc. of phosphate buffer and the freezing and thawing process repeated. The supernate, P-FT-2, was

almost colorless. Supernates P-FT-3 and P-FT-4, obtained in like manner, were practically colorless.

Preparation (b) was allowed to stand at room temperature with frequent stirrings for 1 hour. Centrifugation yielded a deep brown opalescent supernate which was designated P-R-1. The residue was taken up in 10 cc. of buffer and stirred at intervals during a second extraction period of an hour; the pale amber opalescent supernate was designated P-R-2. Third and fourth extractions yielded faintly colored P-R-3 and P-R-4 preparations. The residue from the fourth extraction was taken up in 10 cc. of buffer and left in the refrig-

TABLE 1

Showing the relative activity and nitrogen content of extracts of *P. knowlesi* parasites

EX-TRACT NUMBER	READINGS	P-R	P-FT	S-FT
1	Antigen units/cc.	640	320	80
	Total N mg/cc.	0.32	0.70	0.41
2	Antigen units/cc.	160*	160*	20
	Total N mg/cc.	0.15	0.12	0.05
3	Antigen units/cc.	80*	160	20*
	Total N mg/cc.	lost	0.04	0.02
4	Antigen units/cc.	80*	80	20*
	Total N mg/cc.	0.03	0.03	0.02
5	Antigen units/cc.	20*		
	Total N mg/cc.	0.02		

\* Indicates that the next dilution gave a reading of 3+.

erator over night. This was centrifuged and the supernate designated P-R-5.

Preparation (c) was frozen and thawed as was (a), using 10 cc. amounts of 0.9 per cent NaCl and S-FT-1, S-FT-2, S-FT-3, and S-FT-4 extracts obtained.

Five cc. samples of each of the 13 preparations were used for total nitrogen determinations. Micro kjehdahl determinations for total nitrogen were made on the first two extracts in each series, using selenium as a catalyst followed by distillation into boric acid. The ammonia was titrated with standard HCl, using methyl red indicator to a matched end point. The ammonia from each of the remaining extracts was distilled into 25 cc.

volumetric flasks containing 5 cc. 0.1 N HCl and the distillate made up to volume. Suitable aliquots were nesslerized and compared against a series of standards, using the photoelectric colorimeter.

The relative antigenic activity was determined by titration with a strongly "positive" malaria serum diluted 1:2½. A known negative serum and anticomplementary controls were included. All tests with the negative serum and in the series containing no serum were negative. The highest dilution of each antigen giving a 4+ reaction with the positive serum was recorded (table 1). It is apparent that the phosphate buffer preparations are much more active than the saline extracts. A calculated total of 9600 antigenic units was obtained by 4 phosphate buffer extractions at room temperature, 7200 units by 4 freezings and thawings in phosphate, and 1400 units by 4 freezings and thawings in saline.

The total nitrogen determinations indicate that the first extracts by any method contain more nonantigenic material than subsequent extracts. This finding is in accord with the larger amounts of pigment in the first extracts. The first extracts contain, also, larger amounts of material, presumably protein, which may be flocculated by heating under conditions which do not inactivate the antigen.

### 3. Barbiturate Buffer Extracts

Extracts were prepared by treating fresh or dried parasites with N/10 barbiturate buffer (pH 8.5) at room or refrigerator temperatures for varying time periods. Either method yields active preparations containing 320 to 640 units per cc. Repeated extractions recover additional antigen.

A highly active antigen was prepared by extracting the wet parasites obtained from one monkey first with 200 cc. of barbiturate buffer and then with 50 cc. The combined extracts were dialyzed against distilled water to remove salts, and subsequently concentrated in a semipermeable membrane at 5°C. to 40 cc. Centrifugation yielded a supernate of 1280 units per cc.

### 4. Solutions of Parasites Obtained by Treatment with Barbiturate Buffer and NaOH

Fresh parasites were treated with barbiturate buffer, pH 8.5, at room temperature for one hour. Ten per cent NaOH was added drop by drop, with gentle agitation until the parasites dissolved. A dark brown solution was obtained which was

dialyzed against N/10 barbiturate buffer (pH 8.5) for 3 days at 3°C. Some pigment precipitated during dialysis. After centrifugation the supernate was tested for antigenic content and found to contain 800 units per cc.

### 5. NaOH Solutions of Parasites

Fresh parasites were dissolved in 0.096 N NaOH. The solutions were then adjusted to pH 7.0 with dilute HCl which precipitated most of the pigment and perhaps some other materials. The opalescent pale brown supernate was then concentrated in a semipermeable membrane at 3°C. to approximately 25 per cent of the original volume, a process requiring about 3 days. While antigenic, this preparation was not satisfactory because of its anticomplementary properties.

#### PROPERTIES OF THE P. KNOWLESII ANTIGENS

##### 1. That the antigenic material is a protein-lipid complex is suggested by the following findings:

It has been shown by Morrison and Anderson (1942) that the malaria pigment is hematin. Saline or phosphate buffer extracts of recrystallized hemin (hematin monochloride) are not antigenic when used in precipitative tests or in complement fixation tests. Moreover, the activity of antigens is wholly unrelated to their pigment content. Removal of the pigment from parasite extracts or solutions does not destroy their antigenic properties.

Lipids extracted from wet or dried parasites are not antigenic; furthermore, barbiturate buffer extracts of lipid-free, dried parasites are inactive.

Ether treatment of dried parasites, preliminary to saline extraction by freezing and thawing, reduces greatly or destroys the antigenic properties (Dulaney, Stratman-Thomas, and Warr, 1942).

If wet parasites are first extracted with acetone containing 1 per cent HCl by volume, the residue does not yield antigen by any of the methods which are otherwise productive.

No evidence of a carbohydrate in filtrates of hydrolysed parasite preparations has yet been demonstrated through use of copper reduction methods. The method of Tillmans and Phillipi (1929) for detecting carbohydrates, as modified by Sørensen and Haugaard (1933), and frequently used in protein studies, cannot be applied since strongly positive reactions are obtained when hematin is present.

2. *The antigenic properties of saline, phosphate, or barbiturate buffer extracts are not destroyed by heating in a water bath at 56°C. for 30 minutes, 75°C. for 15 to 30 minutes, and 100°C. for 5 to 15 minutes*

Heating at 56°C. for 30 minutes does not affect the antigen as shown by comparison with unheated samples, but exposure to the higher temperatures reduces the activity by two to three dilutions.

Heating produces flocculation of pigmented material to an extent roughly proportioned to the temperatures and times to which the preparation is exposed and subsequent centrifugation yields an opalescent supernate with amorphous brown deposit. The supernates from extracts heated at the higher temperatures are practically colorless. However, supernates from preparations containing large amounts of pigment are sometimes found to be inactive after heating at 75° to 100°C. which suggests that the antigen is adsorbed on the flocculated pigment and removed with centrifugation.

Neither heated nor unheated antigens are stable indefinitely.

3. *The pigment may be materially reduced by freezing or by drying phosphate buffer extracts*

Freezing will flocculate most of the pigment in phosphate buffer extracts with the exception of the first highly colored preparation. Drying of phosphate buffer extracts by means of a vacuum pump and rehydration by 0.9 per cent NaCl and centrifugation yield almost colorless antigens. These preparations are almost as active, in some cases as active, as the original extracts.

4. *The malaria antigen does not dialyze when left in semipermeable membranes suspended in 0.9 per cent NaCl or water at 5°C.*

#### SUMMARY

1. The antigenic material of *P. knowlesi* parasites is only slightly soluble in saline, more soluble in phosphate or barbiturate buffers of pH 7.8-8.5, and completely soluble when NaOH is added to buffer extracts to give a pH of 9.0 or above. The alkalinity of the phosphate and barbiturate buffers probably account for their efficiency as antigenic extractives. The use of such reagents was suggested by the knowledge that *P. knowlesi* parasites are soluble in 0.5 N sodium carbonate (Sinton and Ghosh, 1934).

2. Phosphate or barbiturate buffer extracts are 6-8 times more active than the saline antigens.

Since 0.5 gm. dried parasites represents the average yield of parasites per monkey it may be estimated that such material will furnish 36,000 to 49,000 antigenic units by extraction with phosphate buffer in contrast to 7,000 obtained by saline extraction. These antigenic units represent, respectively, 9,000, 12,250 and 1,750 doses.

3. Sodium hydroxide solutions of parasites are not satisfactory antigens.

4. It now appears that dehydration of phosphate buffer extracts of wet or dried parasites may offer a highly efficient method of storing malaria antigen. The practical aspects of this method are now being investigated.

5. The present experimental evidence suggests that the antigen is a lipid-protein complex but a carbohydrate factor has not been entirely eliminated.

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# THE REARING AND MAINTENANCE OF A LABORATORY COLONY OF THE BODY LOUSE

G. H. CULPEPPER<sup>1</sup>

From the United States Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine<sup>2</sup>

Received for publication February 22, 1944

The body louse (*Pediculus humanus corporis* Degeer) has been reared in large numbers in the laboratory at Orlando, Fla., for use in investigations of control measures for this insect. The purpose of this paper is to discuss the methods and technique used in rearing and maintaining a large colony of lice. Precise data on the biology of the body louse are not included, since this phase of the work is still in progress and will be reported later. A daily average of approximately 1,200 lice and 875 eggs, or a total of approximately 1,325,000 lice and eggs, have been used in the experiments on control measures during the period of May 1942 to October 1943.

From a small number of lice that were collected from healthy individuals and were fed upon the blood of men that had shown no evidence of disease, it was possible to develop a colony of lice that were free of infection.

The colony is maintained in an incubator at approximately the same range of temperature (86° to 90° F.) and relative humidity (averaging above 75 per cent) as described by Moore and Hirschfelder.<sup>3</sup> The lice are kept on loosely woven patches of woolen suiting cut approximately 1½ inches square with pinking shears. The preferred color is dark blue or black, since both lice and eggs are

more readily visible on dark than on pastel shades. In addition to furnishing favorable footing, loosely woven patches adhere to one another better than smoothly woven or hard-finished materials, which is an aid to the technician in the feeding process.

Each patch of cloth supports approximately 100 adults. Obviously the smaller the lice, the more can be maintained on a cloth patch. The patches with the lice are kept in crystallizing dishes 8 to 10 inches in diameter, as shown in figure 1. Forty to 60 patches containing a total of 4,000 to 10,000 lice, depending on the size of the lice, are carefully placed so they will cover a circular piece of half-inch-mesh hardware cloth cut to fit the bottom of the dish. The wire meshes hold the patches off the bottom of the dish so that the parasites have freedom of movement on both sides of the cloth. Crystallizing dishes are used for the reason that lice cannot crawl up the sides of glassware.

The colony is separated into lots according to age by removing the adult lice from the egg patches every 48 hours to assure a continuous supply of eggs for rearing the colony. The separated egg patches become a new egg lot, and are placed in a beaker of appropriate size, on which the date is labeled so that the time of hatching may be anticipated.

After the eggs hatch, the nymphs are allowed to remain on the same cloth patches until they become adults, when they are transferred to clean patches. When the nymphs become crowded as they increase in size, additional patches are added to the lot. If the lice are maintained on the same cloth during nymphal life, it becomes soiled with molted skins and excreta, which necessitates cleaning. Cleaning is accomplished by first spreading the cloth patches containing the lice in a shallow pan and drying them by use of an ordinary electric lamp. The dry patches are then rubbed lightly together with the hands, and the debris is shaken out while the lice hold to the cloth. In addition to the patches, all glass containers are cleaned at regular intervals.

<sup>1</sup> Grateful appreciation is expressed to R. C. Bushland, who established and maintained the original stock, some obtained by him in Orlando, and the majority collected by W. E. Dove in Washington, D. C. G. W. Eddy was responsible for the louse colony during March and April 1942. N. B. Carson assisted materially by feeding and maintaining the louse colony from June to November 1942.

<sup>2</sup> The data included in this were obtained in connection with investigations conducted at Orlando, Fla., under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Bureau of Entomology and Plant Quarantine.

<sup>3</sup> Moore, W., and Hirschfelder, A. D. An investigation on the louse problem. Minn. Univ. Res. Pub., 8 (4), 86 pp., 1919.



FIG. 1. From 40 to 60 loosely woven patches of woolen suiting containing lice are placed in 8- to 10-inch crystal lizing dishes so that there will be a layer of patches covering the entire bottom of the dish.



FIG. 2. The cloth patches to which the lice cling are systematically placed on the back of the subject, approximately 30,000 to 40,000 lice are fed at one time.

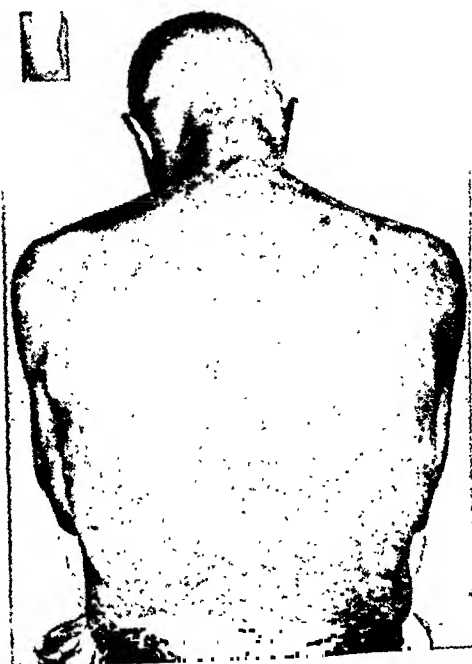


FIG. 3. Dermatitis caused on the back of a host who reacted unfavorably to the bites of the body louse. In a few instances a faint outline of the cloth patch is visible.

The lice are fed on research subjects, who are used also for other assignments connected with the projects. As nearly as practicable, the colony is fed at 12-hour intervals, once in the early morning and once in the late afternoon. The feeding process consists of placing the cloth patches containing the lice systematically across the back of the subject, leaving a space approximately 1 inch wide between the different lots. After the cloth patches are placed on the subject, most of the adult lice immediately go under the cloth and begin feeding. The nymphs do not go beneath the patches so quickly as the adults, and to hasten them ordinary electric lights are held for a short time about 12 inches above the insects. Lice are repelled by the light and heat of the electric lamp and go under the cloth patches, where they come in contact with the skin and start feeding.

As a precautionary measure against becoming infested, the research subject removes his clothing before the lice are fed. After the feeding process has been completed, most of the lice cling to the patches, which are removed from the back of the subject and returned to the crystallizing dish. If lice remain on the subject after the patches are removed, they are taken off with forceps, and as a further precaution a towel is rubbed over the back of the subject to remove any that may have escaped notice.

Lice feed readily, and the entire operation, which includes placing and removing the lice and dressing the subject, is finished within a period of 30 to 45 minutes. As many as 30,000 to 40,000 lice may be fed, as shown in figure 2, at the same time on the back of one subject. As many as 100,000 lice and an equal or greater number of eggs have been carried in the colony at one time. With few exceptions, the research subjects have not been required to feed the colony more often than once per week. In four instances, however, research subjects voluntarily fed approximately 40,000 lice each on two successive days without any apparent ill effect. Two subjects have been observed sleeping soundly during the process of feeding the lice.

There is a wide difference in individual reaction to louse feeding. Some subjects display no visible reaction and experience only a slight, transient sensation of itching, while others develop from slight to conspicuous dermatitis, which persists for 2 or 3 days. Relatively few subjects (fig. 3) who have been employed to feed the lice reacted so severely that they could not serve again in this capacity, but all apparently were normal in 7 to

10 days. Most of the 40 research subjects now employed to feed mosquitoes and lice have been feeding lice at regular intervals for from 6 to 18 months. All apparently are in as good health as when they were originally employed, and periodical physical examinations have shown no ill effects that could be attributed to feeding of the insects.

A method involving a simple device which saves excessive time and hand labor has been used in rearing the large colony of lice. The device consists of a piece of one-eighth-inch-mesh hardware cloth, cut to a convenient size, which is used to combine or to transfer lice to a few or to new cloth patches. To combine or transfer the nymphs or lice, the screen wire is used with a small, shallow pan fitted inside a large pan containing water. Patches of cloth, to which the transfer is to be made, are placed systematically in the small pan. The screen wire is placed over the clean cloth patches, and the patches containing the lice are placed on the screen wire. Electric lamps are arranged about 12 inches above the lice to produce a repellent effect. Water is used in the large pan to produce a cooling effect on the cloth under the wire. A temperature above 105°F. in the small pan should be avoided in transferring adult lice from patches containing eggs, since the eggs are likely to be injured by this exposure.

Rearing and maintaining a laboratory colony of body lice under conditions of high relative humidity has serious disadvantages. If the humidity in the incubator reaches 85 to 90 per cent and remains that high for a few hours, the colony may be affected adversely. This apparently is due to the fact that excreta do not dry readily in moisture-laden atmosphere, causing the cloth patches to become wet and the lice to stick together and to the glass container. Favorable conditions for mass rearing require a temperature ranging from 85° to 90°F. and a relative humidity below 75 per cent. Observations being made on the optimum condition of humidity have not been completed, but sufficient evidence has been accumulated to show that an excessively high humidity will seriously affect the survival and reproduction of a laboratory colony of these insects.

Observations have been made on the mortality and reproductive capacity of lice under laboratory conditions and are being continued. Under the conditions of mass rearing described, the lice live and reproduce in a satisfactory manner to provide a strong colony for subsequent research on control measures.

## BOOK REVIEWS

Received for publication June 15, 1944

*Manual of Human Protozoa*. By RICHARD R. KUDO, D. Sc., Associate Professor of Zoology, University of Illinois, Charles C. Thomas, Springfield, Ill. 1944. Illustrated. Pp. i-ix, 1-125.

This little manual was written for the author's students as a guide to the identification and detection of the protozoan parasites of man. It is very brief and in the reviewer's opinion, of limited value because of the brevity of the descriptions of the organisms considered. However, the author states that it is based upon his notes that were used in an "emergency course offered at the University" and perhaps one would be asking too much to expect a thorough consideration of the subject. The material that is presented is accurate and the volume would undoubtedly be of value to certain students during a course upon protozoology.

The reviewer regrets that Dr. Kudo has seen fit to adhere to the spelling "Entamoeba," instead of "Endamoeba" in describing the amoebae occurring in man, especially in a book intended for American students. He is the only writer in this country, so far as the reviewer knows, who uses this spelling. It would have been much better had he followed the ruling of the International Committee of Zoological Nomenclature, and used the spelling recommended by it as preferable, i.e. "Endamoeba."

CHAS. F. CRAIG.

*Microscopic Technique in Biology and Medicine*. By E. V. COWDREY. Professor of Anatomy, Washington University etc., The Williams & Wilkins Company, Baltimore, Md. 1943. Pp. i-iv, 1-206.

This book is a most excellent compilation in alphabetical form of the techniques employed in biology and medicine in the laboratory and is really a dictionary and encyclopedia of such techniques. The alphabetical arrangement is most useful and the volume should prove of much value to every laboratory technician and research worker. The amount of material considered is great and the method of presentation lucid and as brief as is consistent with the importance of the technique described. The reviewer has had occasion to refer to this work repeatedly and has invariably found the material he desired and his experience will be that of all others who have the good fortune to possess this very useful technical guide.

One of the most valuable features of the work are the references to the original descriptions "of technical methods or to complete discussions" of methods and these references are given with the description of the technique, so that time and labor are conserved.

This book can be cordially recommended to all who have to do with laboratory methods used in biology or medicine.

CHAS. F. CRAIG.

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# ADAPTATION OF PUBLIC HEALTH PRACTICE TO FOREIGN CULTURES<sup>1</sup>

JANET WELCH MACKIE

*From Medical Section, Health & Sanitation Division, Office of the Coordinator of Inter-American Affairs, Washington, D. C.*

Received for publication July 18, 1944

Gregory Bateson has said concerning a far eastern people, "one of the most pressing problems in a world which is becoming increasingly close-knit is that of mutual understanding between peoples with basically different cultures. Each may have in the background of his mind a whole mass of thoughts and values and interpretations of the world which are specifically his. The white man has his notions of competition, sacrifice, fair play, justice, checks and balances, democracy, discipline and so on, the native correspondingly may have in the back of his mind his own interpretations of life, his feeling for symmetry in human relations, his notions of the sacred and the unclean, of gift exchanges and the validation of social events."

This difference of perspective is a powerful factor determining the direction and the progress of development. It has its origin in the cultural inheritance of the people whether that be the product of an ancient highly developed civilization as in China, or the superstition and tradition of a primitive race gradually emerging into the modern world as is the case in parts of Africa.

These differences of perspective become of particular importance in countries where a major part of the population is uneducated, and in countries where the population is composed of widely different racial stocks, each with different fundamental concepts. Such a social organization implies wide differences in beliefs and practices between the individual groups. Foreign ideas must be related to these beliefs and practices if they are to be received with understanding and cooperation. Furthermore, different methods of approach may be necessary to deal effectively with the different racial groups within the same country.

Many of the Central and South American countries present a unique problem with a highly developed modern civilization in immediate

proximity to large, uneducated and wholly undeveloped population groups. Furthermore, extreme variations in climate between the lowlands and the high mountain areas are responsible for a wide range of disease endemicity. These cultural and environmental factors differing widely from one country to another and between different areas within the same country, create certain common problems which affect directly the development of public health practice.

In the initiation of a broad public health program for such regions, therefore, two preliminary surveys must be conducted before detailed plans for operation can be made. These must provide specific answers to two fundamental questions:

1. What are the medical problems?
2. What are the sociological, and anthropological or cultural factors which must be considered in orienting the technique of attack against these medical problems?

The particular problems necessarily will vary in detail from one region to another. However, they will include certain common fundamental factors which must be recognized a priori and which must determine the particular orientation and the detailed method of approach if the operation of a public health plan is to be successful. These basic factors are:

1. The epidemiology of the important diseases is profoundly affected by the social patterns and practices of the people.
2. The cultural background of tradition and belief concerning the cause and cure of disease frequently renders modern public health practices meaningless to the people.
3. Differences in interpretation of and evaluation of life are a barrier to mutual understanding between groups with different cultures.
4. General educational limitations of large portions of the population.
5. Lack of adequate medical care for the population in many areas.

<sup>1</sup> An Address Delivered at a Conference on Inter-American Affairs held by The Inter-American Institute of The University of North Carolina, June 23, 1944.

6. Lack of trained public health personnel.
7. The economic status of certain of these countries, and particularly of the rural areas.

#### GENERAL BACKGROUND OF PUBLIC HEALTH IN CENTRAL AND SOUTH AMERICA

The countries of Central and South America are vast and bewilderingly varied in their climate and geography. There is a wide division in the population between the two main groups—Spanish and *mestizo* on the one hand, and Indian on the other. The Indians again are split into tribes, with totally different social patterns and cultural values, as for example, the agricultural Indians of the mountains, and the forest Indians of the Amazon. These main groups are infiltrated by smaller population streams of European and African origin. The metropolitan areas of these countries display the highest forms of western civilization, wealth, broad culture and cosmopolitan education, with brilliant representatives in the arts and sciences. The great rural areas are marked by the other extreme of isolation, poverty and illiteracy. This contrast is due in part to underpopulation, the lack of adequate communications and the difficulty of transportation.

For example, in Peru alone all types of climate, scenery and vegetation from tropical to subarctic are found. The narrow coastal plain consists of sandy desert and arid hills, interspersed with irrigated green oases. They spread out like the palm of the hand where rivers descend from the Andes to the sea. These fertile areas are agriculturally very rich and support large estates of sugar, cotton, rice and other products. The agriculture is mechanized and the *mestizo* (mixed stock) labor is highly organized. The larger towns of Peru are found chiefly in this coastal belt. The Sierra, or high valley region of the Andes lying between 7,000 and 12,000 feet is occupied by some 2-3 million people of pure Indian stock. They are occupied in agricultural and pastoral activities, with the exception of some thousands in the mines. Peruvian statistics show that the major portion of them are illiterate. Almost half work on large estates and have no land rights. To the east of the Andes lies the Montana—a part of the upper watershed of the Amazon River, covered with dense tropical jungle and probably of great potential agricultural wealth. The population is gathered mainly along the

rivers and is of Indian and mixed stock. In the dense jungle of this area there are also semi-savage forest tribes.

The main urban centers present all the refinements of wealth and modern civilization. The population of these communities consists of the leading group of Spanish stock, an intermediate group of educated skilled workers of mixed stock and a larger group of illiterate and unskilled workers living at a very low economic level.

#### MEDICAL PROBLEMS

The medical problems of South and Central America are extremely varied. Tuberculosis is a major health problem in many countries. Infant death rates are excessively high in others. Typhus, malaria, pneumonia, smallpox, venereal disease, malnutrition and hookworm infestation are likewise of major importance in many areas. The governments and leaders of these countries have recognized the importance of these medical problems. They are dealing with them actively and are desirous of having help in their solution.

It is not possible to present a detailed picture of national public health services in the other Americas. There is a very live and increasingly active interest in public health in all these countries. All have organized National Departments of Health in the capital cities, and are developing with well defined objectives. All display a very keen desire to utilize sound modern principles of public health organization. Like our own services, their evolution is marked by problems of coordination and integration and, as has been the case in the United States in the past, development has been handicapped not infrequently by politics. Over a number of years, United States agencies have given cooperation in various ways for the promotion of health in these countries, especially the Pan American Sanitary Bureau, The Rockefeller Foundation, the Children's Bureau and the Health and Sanitation Division of the Office of the Coordinator of Inter-American Affairs. Through the latter office cooperative health services have been organized with the governments of the other republics, as an integral part of their National Departments of Health. This machinery constitutes a pioneer venture in a new type of cooperative service, uniting the governmental agencies of this and other countries. Technical field parties sent from the United States are supplemented by local personnel, just as funds and

equipment are supplied by both the United States and the participating Republics.

The major present problems are the provision of adequate services and sufficient trained personnel to provide preventive care for the masses on a country-wide basis. In some countries, public health organization is still directed only to the control of certain specific communicable diseases which have assumed importance in particular areas. The more modern concept which implies localizing all health activities for a community in a Health Center, administered by a full-time public health doctor, which provides complete well-balanced services through care of the family unit, is not generally understood nor applied. Although the Inter-American Cooperative Health Service is assisting in the development of such modern Health Centers, these are located principally in the larger cities. None of these countries provide adequate public health services for their vast rural populations. Some have practically none outside the main centers of population and even then the services are frequently insufficient.

These factors of race, culture and beliefs, the general level of education and the economic status of the population combine with the existing deficiencies of public health philosophy, organization and practice to produce innumerable specific local problems each of which may require a different technique for solution. Some of the varied combinations of these factors are indicated by the following illustrations:

#### 1. Lack of adequate medical care

Medical care of the people has to be a function of the health services in many areas of these countries. Provincial areas with rural populations at extremely low income levels cannot provide adequate incomes for private doctors, nor even purchase essential medicines if the doctors' services are furnished free of charge. Thus, in the Amazon Basin, where Inter-American cooperative health work is being undertaken, the construction of small hospitals or centers with beds is the first and immediately urgent step in the community health program. Provision of medical care is essential because of the great amount of sickness. It is of value also in gaining the confidence of the people and providing the opportunity for imparting elementary instruction in the prophylaxis of the important communicable diseases.

#### 2. Disease problems related to the local beliefs and practices and to the concept of cause and cure of disease

The epidemiology of a disease is modified by the sociologic pattern of the community as well as by the variations in physical environment.

Special sociologic problems may greatly affect the epidemiology of a disease in a particular community. Thus in Bolivia, there are two areas—Cochabamba, with its broad valley at 9,000 feet, and Chulumani in the lower ranges of the eastern Andes at elevations of 3,000 5,000 feet. Both areas have a malaria problem and both have the same vector: *A. pseudopunctipennis*. In Cochabamba, the mosquitoes go to the people from breeding places in irrigation streams and reservoir water. In Chulumani, the men and boys go to the mosquitoes because of the practice of guarding ripening crops in the valleys through the night from thieves and sleeping by the streams. So marked is the increased exposure to the bite of mosquitoes through this practice that there is a much higher incidence of malaria among the boys and men than in the girls and women, since the latter do not leave the villages on the hills.

The empiric knowledge of even an illiterate people concerning the epidemiology of a local disease may determine the continued endemicity of another, and it may defeat efforts for control. In Peru, the western valleys of the Andes leading down to the sea have two major health hazards, malaria at the lower altitudes and Oroya fever in the 800–3,000 meter altitude. The range of the malaria vector *A. pseudopunctipennis*, extends well up into the Oroya fever areas. If mosquito control measures could be extended into the upper valleys, malaria unquestionably could be eliminated. This is not practical, however, since the people have learned that remaining in the upper valleys over-night is attended by grave risk of acquiring Oroya fever, a highly fatal disease transmitted by a night-flying black fly, a species of *Phlebotomus*. In consequence the anopheles mosquito, despite control measures in the lower valleys, is constantly spreading downward from the uncontrolled higher altitudes.

Some knowledge of the customs and beliefs of the Indian peoples is a prerequisite for success in establishing a maternal and child health program. When in Bolivia, I was told that infanticide was not uncommon among the large

Indian populace in La Paz, and that abandoned infants were thrown down into the river ravine. However, reading later a study of the Aymara Indians, I learned that it was not the custom of the tribe to bury stillborn infants. They were thrown into a nearby river or Lake Titicaca. An understanding of this would alter the apparent significance of the problem for the Maternal and Child Health worker.

The concepts of the Indians concerning the causes and cure of disease must be considered seriously in the institution of clinic services. Customary procedures may need to be extensively modified to meet these beliefs and to retain the confidence of the people. The individual medical problem involves much more than the technical procedures of diagnosis and treatment. Native medical art makes strong appeal to the emotions in contrast to our scientific procedures. Our detached attitude toward the patient and the disease may easily be misunderstood. To them the manner of treatment is as important as the method. Among many different culture groups, including the Indian, the treatment of a sick person is a "community act," not an individual matter. The family and all who are present take part in the ceremonial of treatment and believe that, by the mere fact of their presence, they contribute to influence the course of the disease and to aid the patient. In such a society insistence upon private examination and application of unmodified conventional methods of therapy will arouse both fear and distrust.

Sociological factors, however, are equally operative in health work in the metropolitan areas of these countries. The social values of the leading group, the type of education and the concept of woman's place and functions in society all have had marked effect on the development of public health work in the past and call for special orientation of the program. Some of these attitudes have served to disparage nursing, which has been regarded as servants' work, while encouraging community work through social service. The result is that in many of these countries, there are no public health nurses of professional standing. Clinic work and follow-up home visiting are undertaken only by social workers.

There is a further psychological factor of the highest importance in the development of a public health program in new areas. This is the personal relationship between the field worker

and the people. Among undeveloped population groups human relationships are all important. In a highly educated society, on the other hand, the difficult or unsympathetic personality may be accepted because of his professional contributions. This is not the case, however, in a backward and unsophisticated population. Selection of individuals, therefore, must be made with care, and frequently they must receive detailed instruction concerning the society with which they are to work. Without direct and sympathetic personal relationships a plan however desirable will not win the cooperation of the people.

### *3. Educational and economic factors*

In the countries under discussion, there is great disparity between the need for and the supply of well-trained personnel. Urban centers require more than are at present available, and professionally trained people naturally do not seek employment in backward areas, isolated by mountains and jungle. The economic status of many areas likewise precludes the employment of adequate numbers if they were available. These two factors necessitate no inconsiderable modification of United States techniques which are based upon adequate full-time professional public health personnel, ample funds and an educated public. They call for specific orientation of public health work to an education program for the preparation of public health personnel and for widespread health education of the people. They call also for simple and relatively inexpensive programs of preventive care, which are within the financial ability of the state to provide.

It has been demonstrated in many areas of the world that effective public health programs can be carried out at varying levels of expenditure when adapted properly to meet the local situation, provided the prior understanding and cooperation of the community is obtained. Such cooperation implies a large degree of voluntary participation by the people. Obviously such a program must be limited in scope and its objectives must not exceed the capabilities of the community. Furthermore, it must be linked directly to the development of other social and educational activities if it is to become firmly established.

The need for health education is everywhere apparent in urban and rural areas alike. In many regions also there are signs that the people

are aware of their need for health protection measures but are uninstructed and ignorant of how to achieve them. Control measures accepted by a community without instruction as to their purpose are seldom effective, as the following illustration demonstrates. There is a vast, little-known jungle region along the Abuna River, one of the headwaters of the Amazon on the borders of Bolivia and Brazil. The entire population scattered along the banks is extremely isolated and has been without doctors or medical care other than drugs obtained from the store in the larger settlements. Malaria is prevalent throughout the area. The houses are built of wood, supported several feet above the ground by posts. The floors are of split palm with many cracks and spaces opening to the exterior. Although mosquito nets, supplied by the local stores, are widely used, they are of no value since at night the people do not close them at the bottom and mosquitoes enter freely through the openings in the floor.

The different evaluation of life due to different cultural backgrounds is a profound problem for the foreign health educator, because certain factors which we know to be detrimental to the welfare of a group are regarded by them as an asset. What, for example is the health education approach to the following? Goitre is a major health problem in some of the high valleys of the Andes. In some districts, the enlargement of the neck due to this condition is regarded as a physical asset and girls with thyroid enlargement obtain husbands more readily than those without.

#### METHOD OF APPROACH TO THESE PROBLEMS

These are the problems in broad outline. They are the problems of all countries that have large rural areas and a large proportion of the population existing at low economic and educational levels.

The institution of public health practice in such regions is not just the establishment of administrative machinery. The initial step is education as to why public health administration is needed and how it can benefit the people individually. The problems of these areas demonstrate that public health practice is nothing more nor less than education for living through the translation of technical medical knowledge into community activity.

Western medicine has provided a blueprint of public health organization and administration

which is the admiration of the world. It has provided guiding principles for health programs in many countries. It has not developed techniques for translation of this technical knowledge into practice, successfully and completely for population groups at low socio-economic levels. This is the outstanding public health problem of the world today. Inherent in this question of mass health education is the problem of the provision of public health personnel trained to give health instruction while providing technical service.

The solution lies in a change of emphasis in public health education at professional and postgraduate levels. Curricula must be reoriented to provide instruction for personnel at all grades so that they may become an effective force in direct health instruction of people who must be taught to protect themselves.

A new orientation of professional public health personnel is required in order to give greater emphasis to their function as educators and instructors. Instruction should include orientation in the cultural and environmental factors characteristic of the area to which they will return so that they may translate effectively to the people the scientific facts and methods which they need to know. In post-graduate work public health students should be taught that one of their principal activities on their return to their own country will be to train others. Strategic posts in the public health services of Latin America should be filled in so far as possible by individuals who have had formal professional training, and these officers must function not only as administrators but as guides and teachers for less qualified staff members. Such instruction can be given effectively only in the health center, not in an academic classroom.

Real understanding of this need, and suitable preparation of the top-ranking professionals to give practical instruction to their immediate subordinates in the course of their professional activities as public health officers, health center directors, sanitary engineers, bacteriologists, or professional public health nurses, could in a few years greatly improve the level of operation of the rank and file of the health service—without the cost of special courses and high salaried educators.

The preparation locally of technical public health personnel is of primary concern to public



health departments where public health doctors and other public health professional workers are so few in relation to the need.

There are divergent opinions regarding the development of public health training in foreign countries. One group considers that professional training at the highest levels of education alone should be undertaken with direct service by professional staff. This service with high professional standards, they contend, will gradually spread from the main urban centers out to the rural areas. However, at the rate at which thoroughly competent individuals are trained in most of the countries under discussion, it will be many years before there can be a country-wide service on a professional basis. Furthermore, the cost of this type of public health service would be prohibitive for most areas.

The second group considers that professional training must be stimulated to the utmost at the urban centers and direct services at professional levels rendered where possible. In addition professionally trained public health personnel should be used for instruction and supervision of a much larger group of technical workers who will actually carry out the direct service to the community. Such a plan can be accomplished at a cost within the resources of the poorer provincial areas.

Preparation of technical workers at different levels of education adjusted to the general development of the area has been undertaken in different parts of the world with varying success. Such training can be effective in reducing infant mortality and disease morbidity rates when it is carefully planned, conducted by well qualified professionals, and when a regular system of refresher courses is provided for continued instruction of the workers. The movement for the reconstruction of China, inspired by Dr. Yen, has evolved a system of rural public health, the key to which is instruction of the people to do their own public health work. Handicapped by extreme poverty, no elaborate scheme of preventive care could be considered. Handicapped also by an extreme dearth of professional doctors, the people are shown how to guard their own health in their own village by their own village health worker, who is trained in certain specific preventive care activities. Serious medical conditions are passed on to a doctor in a subcounty health center. This in turn is served by the large county health center which has hospital beds and

trained doctors and nurses. Thus the system is economical of professional personnel and low in cost. Discussing the training of these health workers, Dr. Yen relates how Chinese doctors graduated in public health in the United States had to be reeducated and taught methods which are suitable for Chinese villages.

Because of the great amount of preventable disease in many areas of Central and South America, community workers with sound medical technical training are essential to ensure success of a community health program. Health education through schools and other agencies must be supported by medical workers in the community. The preparation of these health workers at elementary levels of general education is a highly specialized problem requiring different techniques of approach, in different regions and for different races. The individuals to be trained must be responsible members of the community in which they are to work and they should have had the best education that is available in the area. They have two functions which are completely integrated.

a. The performance of certain technical medical activities within the community. The male worker may have such duties as vaccination, registration of births and deaths, certain sanitary duties and, it may be, the giving of certain simple treatments. The woman worker has responsibilities in supervision of and instruction in home sanitation, infant care, nutrition, simple home care of the sick and home delivery service.

b. They must, in carrying out their technical duties, at all times give health information and guide the people in developing their own health protection.

Their major function then, is *instruction through demonstration*, or "showing how while saying why." For the performance of these duties, sound technical training is required, based on an understanding of the cause of disease and the principles underlying sanitary and hygienic practices.

Throughout the course of instruction for these workers, emphasis must be placed on the techniques for imparting technical knowledge to the uneducated and uninstructed. This requires not only the careful integration of Health Education with every phase of the instruction but likewise the active assistance of health educators in the training program.

METHODOLOGY OF ELEMENTARY HEALTH INSTRUCTION OF THE PEOPLE, WITH PARTICULAR REFERENCE TO THE MATERNAL AND CHILD HEALTH PROGRAM

Health departments have realized that it is useless to plan a public health system requiring an army of full-time workers even at the non-professional level in the areas of which we are speaking. The people must be taught to protect themselves. This can be accomplished most rapidly by the introduction of health education in the schools and an approach to the adult population through local leaders. There are men and women in every community whether in urban street or tribal village, who are potential leaders and who influence the thought and action of their neighbors. They can become leaders in health activities when their interest has been aroused. The preparation of these local leaders has a twofold objective; information for themselves and preparation to pass it on to their neighbors.

At the present time, therefore, there is great need throughout the world for the development of techniques for the presentation of medical knowledge in much simpler fashion to groups at lower levels of general education. An incentive sufficiently strong to alter life-long habits can only be created by genuine understanding of the causes of disease and of the reasons underlying measures for disease prevention. The attempt to change the daily habits and attitudes of a lifetime is a stupendous task at best. It cannot be successful unless the teaching techniques are carefully adapted to the educational standards and the cultural background of the particular population group. Interest must first be aroused, which is no easy matter since people have no desire to change lifelong ways of doing things. Next, the truth must be convincing and, finally, the importance of the facts must be brought home. Unless people are convinced of the truth and of the importance of the new ideas, knowledge will never be translated into practice. We may be interested in another society's beliefs and practices, but until we are convinced of their truth and of their importance to us we have no desire to adopt them.

Medical scientific facts can be grasped and will be accepted by the student without previous preparation in the biological sciences if they are presented in the form of demonstration and not as didactic lectures. The latter merely becomes the

teaching of a new set of rules imposed from without, the reasons for which are not understood. While there is much in the fields of biochemistry, physiology and nutrition, for example, which cannot be understood without preparation in the basic sciences, yet the fundamental fact of biology—the *living cell*—can be clearly demonstrated even to wholly untrained individuals. On this foundation the essential concepts of reproduction, the union of the ovum and spermatozoon, the development of the embryo and the growth and function of the body can be built in non-technical language. Instruction may then be amplified to explain the requirements for normal nutrition, the production of waste materials, and the effects of disease upon the body. Further, after demonstration of the living cell these students can obtain a surprisingly clear conception of bacteria, their relation to disease and understanding of some of the important means of entry into the human body. Thus the microscope becomes the most essential piece of instructional apparatus for elementary courses—useful even for health education of lay groups. Understanding of microscopic life can be attained only by visual proof, not by faith.

Because of educational limitations the presentation of these new scientific concepts must be based upon the known field of the students' own experience, if they are to be understood and to be convincing. The teaching of micro-biology is planned to give the student an understanding of the existence and activities of micro-organisms, their relation to health and disease, and hence the need for sanitary practice in daily life. This instruction must begin with a fact within the general knowledge of the student. This may be the knowledge that eyeglasses enlarge the size of letters. Following this, the principle of magnification can be further illustrated by demonstrating the greater magnification of familiar objects when seen through a powerful reading glass. Then follows the suggestion that more powerful lenses might enlarge even invisible things so that they become visible to the eye. This leads directly to the microscope through which the students soon learn to look. Under the low power lens invisible objects are demonstrated which are within the knowledge of the student—dirt on a fly's leg, the stamen of a flower, dust on a hair from the head, the detail of a mosquito's wing. There follows next a discussion of the possibility that some invisible objects might have life, things smaller than mites or maggots. Water from a local

stream, pool or pot of standing water can be used to demonstrate amoebae and other forms of living microscopic life. The fact that boiling, strong sunlight, and certain drugs destroy such life can also be shown. Growths of bacterial colonies can be prepared with simple equipment, incubation at room temperatures being easy in the tropics. Bacterial colonies can be grown from flies' feet, from droplets from coughs and sneezes, from finger prints, from teeth, dust and other common familiar sources.

An introduction is then made to the concept that certain micro-organisms cause disease and come directly or indirectly from a case of the disease. The meaning of "infection" and effects of invasion of the body by micro-organisms can be explained. The fact is then discussed that all bodily discharges are dangerous since they may contain disease germs, hence the need for sanitary practices in disposal of any bodily discharge. It is possible also to present convincingly the need for personal hygiene and health habits to prevent conditions suitable for the life and growth of bacteria. From this general introduction there follows logically the need for greater care and cleanliness when dealing with the sick.

Parasitic diseases are of great importance in unsanitated areas and a comprehension of such conditions is made easy by the visual demonstration of parasitic worms and eggs, parasites in blood smears, actual insect vectors and visits to breeding places. In the teaching of preventive measures no opportunity should be missed to demonstrate various aspects of the disease by these procedures—the demonstration of parasites in blood films, micro-organisms and eggs in discharges, and the vectors of disease.

This laboratory method of presenting the background of microscopic life is essential for adequate understanding of the basic principles underlying procedures which practical "non-professional" health workers are expected to carry out. It is likewise invaluable for the creation of a sound understanding on the part of the lay public of the methods for disease prevention which they must undertake for their own protection.

I found in rural Maternal and Child Health Programs in Africa that village women, many with little or no education, quickly learned to look down a microscope, after the concept of magnification of small objects by means of the ordinary spectacle and hand lens had been

demonstrated. The introduction to living microscopic organisms was made by demonstration of water which they drew from the local stream. They found this extremely interesting, indeed, exciting—and from that point on, the concept of the microbic origin of disease became intelligible to them. Without proof and demonstration of this kind their old cultural beliefs were impossible to break down. Following from this concrete introduction to bacteria, however, demonstrations of sanitary practices in the home became of interest and the reasons for them were easily understood.

Finally, when facilities are available, group visits should be made to water supply installations, sewage disposal plants, sanitary slaughter houses and markets, mosquito control projects and other public health activities which may be used to stimulate interest in methods for protection of the health of the community as a whole. This leads immediately to explanation of the women's responsibilities for proper utilization of these facilities and emphasis upon protection of individual water supplies, the need for proper sewage and garbage disposal and the protection of family food supplies.

The attitude of the women determines the outcome of any effort to alter the social environment of communities when public health programs are being undertaken for the first time. They exercise complete control of the children, of the activities within the home and they do the marketing and the preparation of food for the family. The educational phases of such a program therefore, become of great and immediate importance, and from the outset must be directed to instruction of the mothers. It is only after such an introduction to the dangers of the unsanitated environment and to fundamental preventive measures that instruction concerning the control of communicable disease, infant care, nutrition and other aspects of the mother's responsibility for the health of her family become intelligible and convincing. Moreover, an effective Maternal and Child Health program can be initiated only with the complete understanding and cooperation of the mothers in the community. This is the most urgent need in many regions of Central and South America, since excessive infant and childhood mortality prevents the development of the countries by maintaining underpopulation and lack of development of natural resources.

I have dealt only with health education as a

direct public health function. It is essential for success, however, in undeveloped areas that health instruction initiated by public health activity be a part of cooperate planning with educational, agricultural and other social services for a balanced development of the community and for improvement of standards of living. Such cooperate planning should be undertaken at first on a demonstration basis.

#### CONCLUSION

The fundamental public health problem in Latin America and in many other countries is the development of economical and effective methods for extending the protection and services provided for the large cities to include the large and at present unprotected rural populations.

The problems existing in these regions demonstrate that in any area of the world public health practice is merely education for living through the translation of technical medical knowledge into community activity. Health education of the people in backward areas, therefore, becomes the only effective substitute for the protection provided by the state in the more advanced areas. It is the only practicable financial solution and it is highly democratic in its distribution of responsibility throughout the community. When applied effectively its results may be dramatic. Increased population, more rapid economic development, an increase in funds available for public health purposes, and an increasing demand by the people for state and university development of public health administration and field practice.



# THE BLOOD PRESSURE OF THE CUNA INDIANS

B. H. KEAN<sup>1</sup>

*From the Board of Health Laboratory, Gorgas Hospital, Ancon, Canal Zone*

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The Cuna Indians (or San Blas Indians) are a group of approximately 15,000 native Panamanian Indians who live on the San Blas Archipelago, a string of tiny islands which stretch along the Atlantic coast from the northern part of Colombia to within 100 miles of the Canal Zone. Most of these islands are but a few miles from the mainland where many of the Indians have small plantations consisting mostly of coconut trees which they visit daily. Until a century or two ago the Indians lived on the mainland, but their death rate was very high, possibly because of malaria. They therefore moved to the salubrious, windblown islands which are now heavily populated.

The Indians are short, stocky, dark people, four and one-half to five feet tall, with smooth, jet black hair, round faces, high cheek bones, broad noses, large, almost barrel-shaped chests, and short extremities. Fishing and minor agricultural pursuits on the mainland provide their food which consists mostly of coconut milk, fried or boiled green bananas, and fish. Other items in their dietary include plantain, sugar cane, cocoa, yams, yucca, mangoes, oranges, papaya, pineapple, and, rarely, pecarry, fowl, or deer. The Indians have had little contact with the rest of the Republic and have remained practically a "pure race." Some of the youths now, however, travel to the Canal Zone where a few stay for years. The Indians appear to be in excellent health, although three cases of tuberculosis were encountered. These studies were made on the western group of islands which were visited during a ten day expedition. These islands were Nargana (including Corazon de Jesus), Cucti (Rio Azucar), Urganti (Rio Cidre), Carti Sugtup (Carti Cangrejo), Carti Yantup, Mirya Ubigantup (Soledad), and Carti Tupile.

## METHOD

In obtaining the blood pressure readings an attempt was made to follow the recommendations of the American Heart Association (1). All read-

ings were taken on the left arm with the patient and physician seated. Mercury sphygmomanometers with standard-sized cuffs were used. After the patients were seated, a short time was permitted to elapse before the readings were taken. Two auscultatory readings were taken on each individual. If the systolic readings were not within



FIG. 1. CUNA INDIAN WOMAN

6 millimeters of mercury of one another, successive readings were taken until two readings approximated each other. The blood pressure assigned to each individual was the average of the last two readings (or the *only* two readings). In a few instances the foregoing technique was only approximated. The systolic blood pressure was read at the sudden appearance of a clear sound as the mercury column dropped slowly (first phase).

<sup>1</sup> Captain, Medical Corps, A.U.S.

Diastolic blood pressure was read at the initial replacement of the clear sounds by a muffled sound (fourth phase).

#### THE DATA

A total of 915 readings was taken on 408 Indians. In tables 1 and 2 are recorded summaries of the data on 407 Indians. The only blood pressure

females were encountered. No definite tendency for the blood pressure to rise with age could be demonstrated. (The figures in the age group over 65 represent merely data on 13 Indians. Although the average systolic blood pressure in this group was a few millimeters higher than in other age groups, the diastolic blood pressure did not show a comparable rise.)

TABLE 1  
*The blood pressure of Cuna Indians by age groups*

	AGE 16-25	26-35	36-45	46-55	56-65	66 PLUS	TOTAL
Total number of Indians.....	90	115	90	64	35	13	407
Average systolic pressure.....	107.9	104.4	103.7	104.5	105.3	109.4	105.2
Average diastolic pressure.....	70.9	69.0	69.4	70.0	66.0	66.7	69.3
Number of males.....	54	70	63	45	21	10	263
Average systolic—males.....	109.7	104.4	103.3	104.0	105.5	108.1	105.4
Average diastolic—males.....	71.5	68.7	69.4	69.5	66.6	66.3	69.3
Number of females.....	36	45	27	19	14	3	144
Average systolic—females.....	105.1	104.3	104.7	105.6	105.0	113.7	105.0
Average diastolic—females.....	69.9	69.3	69.2	71.0	65.4	68.7	69.3

TABLE 2  
*Distribution of Cuna Indian blood pressure groups*

NUMBER OF INDIANS	SYSTOLIC (MM.)									TOTAL
	66-79	80-89	90-99	100-109	110-119	120-129	130-139	140-149	150 plus	
Males.....	9	19	70	56	55	41	11	2	0	263
Females.....	3	9	41	43	30	11	7	0	0	144
Total.....	12	28	111	98	85	52	18	2	0	407

NUMBER OF INDIANS	DIASTOLIC (MM.)							TOTAL
	40-49	50-59	60-69	70-79	80-89	90-99	100 plus	
Males.....	2	36	94	95	35	1	0	263
Females.....		16	64	50	11	3	0	144
Total.....	2	52	158	145	46	4	0	407

readings not included in these tables were those of a 60 year old male whose blood pressure was 180 mm. of mercury systolic and 30 mm. diastolic. He had a history of a chancre acquired in the United States when he was a seaman aboard a sailing vessel, inadequately treated syphilis, and all the physical signs of insufficiency of the aortic valve.

The average blood pressure of the entire group was 105.2 mm. systolic and 69.3 mm. diastolic. No significant differences between males and

A striking finding is the total absence of hypertension. Not a single Indian had a systolic blood pressure over 150 mm. or a diastolic pressure over 100 mm. Two males had systolic pressures of 144 mm.; all other systolic readings were under 140 mm. Four Indians had diastolic readings between 90 and 99 mm.

As can be seen in table 2, a large proportion of the Indians had "hypotension." Forty had systolic blood pressures under 90 mm. and 54 had diastolic blood pressures under 60 mm.

## DISCUSSION

Discussions of the literature on the anthropathology<sup>2</sup> of blood pressure have been provided by Shattuck (3) and Kean (4) and need not be repeated here. It is our impression that the blood pressures of the Cuna Indians represent the normal arterial tension of a healthy people and may be construed as evidence that normal blood pressure does not rise with an increase in age. We are in agreement with the opinion of Robinson and Brucer (5) that the standards of normal blood pressure must be revised downward, and disagree with the views of Master, Marks and Dack (6) that mild or moderate hypertension "can no longer be considered abnormal" at age 40 years and over. Of course our series of cases is too small to permit definite conclusions.

In a previous paper (4) the striking differences between the arterial blood pressure of West Indians (Negroes) and Panamanians (mostly mestizos) living on the Isthmus of Panama have been reported. The incidence of hypertension was seven times greater in West Indians than in Panamanians. These figures, confirmed by Marvin and Smith (7), suggest that there is some fundamental difference between these peoples which is responsible for the development of hypertension in West Indians, its scarcity in mestizo Panamanians, and its apparent absence in Cuna Indians.

<sup>2</sup> Anthropathology is a term recently introduced by Lewis (2) meaning comparative racial pathology.

## ACKNOWLEDGMENT

The author wishes to express his gratitude to Brig. Gen. M. C. Stayer, Chief Health Officer, Canal Zone, for permission to make this study, to Col. R. D. Harden, Superintendent of Gorgas Hospital for his daily advice during the expedition, and to Dr. Henry W. Kumm of the Rockefeller Foundation who kindly provided facilities for making the trip.

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# HEALTH STATUS OF THE MARSHALLESE

## A PRELIMINARY REPORT

LOUIS SHATTUCK BAER AND RALPH R. ALLEN<sup>1</sup>

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### INTRODUCTION

The following paper is the result of a survey made under the direction of Capt. R. F. Sledge, MC, USN, whose suggestions and encouragement were of great help to us. The observations recorded below were made by the authors during the months of March and April 1944. The total population of the islands and atolls visited was approximately 4500; we examined 1100 of this total.

No accurate morbidity percentages are available as yet. Those quoted in this paper are approximations. We are at present engaged in making a more thorough survey of the diseases found among these people. This will be the subject of a later paper.

The number of our laboratory studies was limited by the short time we had ashore on the various islands visited, and the extreme difficulty of getting equipment ashore across the reefs surrounding these islands.

### GENERAL

The health of the natives was fair. In general it was poorest among those on isolated islands surrounded by nearly impassable barrier reefs. It was best among those who lived on islands where the Japanese had had military hospitals and military medical personnel.

Since the onset of the war, with consequent disruption of Japanese civil administration, the health of the Marshall natives has suffered. The single most important factor has been the exhaustion of arsenical supplies used in the treatment of yaws.

**Yaws.** Yaws is the most important disease requiring immediate attention. Though the incidence varies in different atolls, it is conservative to say that at least 75 per cent of the children under 10 years have active yaws. The percentage of adults having clinically active yaws is lower than

that in the children; however Kahn tests done on 120 adults were positive in 92 cases.<sup>2</sup>

All the major lesions attributed to yaws were seen among these people. We saw every type of cutaneous manifestation, ranging from generalized maculo-papular lesions to the single large framboeside. Numerous cases of "crab yaws," juxta-articular nodules, active and quiescent periostitis of the long bones, (particularly the tibia), degeneration of the nasal septum with consequent deformity of the nose (gangosa), n'Gonde, and destructive osteomyelitis of the phalanges were among the lesions seen by us. The cutaneous yaws lesions occurred most commonly on the exposed portions of the lower and upper extremities. In children peri-anal condyloma and ulcerating lesions in the angle of the mouth with secondary involvement of the chin and neck, were common. Several cases with dry scaling depigmented lesions of the dorsum of the hands and feet were seen which clinically resembled Pinta. These cases rapidly responded to arsenicals.

**Gonorrhea.** Depending on the atoll, anywhere from 60 to 90 per cent of the adult males gave a history of having had one or more attacks of purulent urethral discharge. Most of the natives call this "siplis," though some of the better trained native medical practitioners recognize it as gonorrhea. The use of the misleading term "siplis" is the reason for the incorrect reports that a large percentage of the Marshallese have syphilis.

The per cent of active gonorrhea remains to be determined. Approximately 5 per cent of the male population of some atolls have voluntarily applied for treatment of urethral discharge which on laboratory examination was proven gonorrheal in origin. Gonorrheal vaginitis has been seen in six women, and two acute cases of gonorrheal salpingitis have been noted. Gonorrheal ophthalmitis occurs on those islands where

<sup>2</sup> Kahn tests done by Capt. L. L. Swenson, M.C., A.U.S.

<sup>1</sup> Lieut. U.S.N.R. and Lieut. U.S.N.

there are no native medical practitioners or when his supply of silver solution has been exhausted.

*Other venereal diseases.* 1. *Syphilis*. No chancre has been seen; neither have we noted any luetic alopecia or evidence of neuro-syphilis. One case of aortic regurgitation was discovered in a man of fifty; another man was seen who had been paraplegic for five years, presumably as the result of two cerebral hemorrhages. These cases probably represent instances of cardiovascular lues, but they need further study. Many gummatous lesions of the skin have been seen. It is impossible to say whether these are due to yaws or syphilis. However, due to the high incidence of the former it is our opinion that the lesions are caused by *Treponema pertenue*.

2. *Chancroid*. This disease occurs; so far two active cases have been noted.

3. *Lymphopathia venereum* (lymphogranuloma inguinale, tropical buboe). Two far advanced cases of this disease have been seen. The worst was in a woman thirty years of age who was suffering from multiple peri-rectal abscesses and rectal strictures. Approximately 15 to 20 per cent of the adult males have scars of previously draining bilateral inguinal buboes. It is planned to conduct a Frei antigen survey in the near future.

4. *Granuloma inguinale*. No proven case of this disease has been seen. Skin scrapings from one suspicious lesion failed to show Donovan bodies.

*Tuberculosis*. Pulmonary tuberculosis exists among the Marshallese, but it does not appear to be common. In a rapid fluoroscopic survey of 120 adults only one definite case was discovered. Several persons in the late middle age group suspected of having pulmonary tuberculosis on basis of history and physical examination proved on further study to have bronchiectasis. We have seen one native die of tuberculous peritonitis and have observed one instance of undoubted tuberculous laryngitis.

We plan to conduct a tuberculin survey in the near future, getting chest x-rays on the positive reactors. Eventually a photofluorographic survey should be made.

*Dengue*: We confirm previous reports that this disease occurs frequently among the Marshallese. It is most prevalent from July to October. The vector, *Aedes aegypti* is present on all atolls.

*Dysentery*. Both bacillary and amebic dysentery occur. Among children the former is one of the

most frequent causes of death. From native medical practitioners we have obtained histories of what we assume to be small epidemics of bacillary dysentery. There is ample opportunity for it to spread, as flies abound in every village.

*Leptosy*. This disease occurs among the natives. We have seen five cases and have obtained a history from two children that their respective parents were in the leprosarium at Jaluit.

*Skin diseases*. Tinea cruris, tinea versicolor, tinea circinata, tinea capitis, and tinea imbricata were all seen. The infections are more severe than similar infections in the temperate zones. Scabies and impetigo contagiosum occur frequently among the children.

*Intestinal parasitism*. On the basis of a hurried preliminary survey of 100 stool specimens it can be said that these people are not heavily infested with helminthic parasites. Neither does there appear to be an unusually high carrier rate for *Endamoeba histolytica*. Nonpathogenic intestinal flagellates are common. A thorough stool survey is planned for the near future.

*Typhus*. Endemic, flea borne, murine typhus does occur. We have seen three cases substantiated by agglutinations in high titre of *Proteus* OX<sub>19</sub>. There is no historical evidence that epidemic typhus exists. So far we have seen no case of infestation with the body louse among the natives. Tsutsugamushi fever apparently does not occur; we have not found its vector on these islands.

*Plague*. There is no evidence of plague. The commonest rat on these islands is *Ratus exulans concolor*, a small brown rat. In three trapped specimens so far examined we have found fleas of the *Ctenocephalus* variety but no *Xenopsylla cheopis*.

*Malaria*. No anopheline mosquitoes have been found and no evidence of clinical malaria has been noted.

*Dental caries*. This is a common defect in a large majority of adults. The most frequent type of cavity was a central one of the molar teeth.

*Vitamin deficiencies*. The chief items of native diet before the American occupation were fish, coconut, breadfruit, pandanus, papaya and banana, supplemented with rice, flour, biscuits and canned salmon purchased from the Japanese. With the onset of war the native supplies of the latter have been exhausted and due to lack of fish hooks their supply of fish has been low. During the dry season there frequently is not enough water

for drinking or cooking, and at this time on the poorer islands the natives are reduced to eating coconuts and little else. It should also be remembered that bananas, breadfruit and papaya are all seasonal.

We have noted an unusually large number of anterior peroneal nerve palsies. At first we thought these were the result of old poliomyelitis deformities, but since that time we have seen two acute cases of definite peripheral neuritis involving the anterior peroneal nerves and we now favor the view that dry beri-beri is the cause for these palsies. Only an occasional tongue will show pre-pellagrous lesions; no clinical pellagra has been seen. One case of cheilitis probably due to riboflavin deficiency has been noted. Many natives have gums that bleed easily, are spongy and hyperplastic.

*Ocular pathology.* Pterygium is very common; probably 50 per cent of the adults are afflicted. Senile cataracts are frequent among the aged and are not uncommon among the middle aged.

*Degenerative diseases.* Hypertrophic arthritis, arcus senilis, senile cataracts, arteriosclerosis, diabetes, dorsum rotundum, and senile keratoses are all seen. An occasional case of benign hypertension (asymptomatic and without cardiac enlargement), has been noted. It is certain that the native's easy mode of life, their perfect climate and the abundance of "thalasotherapy" does not protect them from the degenerative diseases of mankind.

*Streptococcal diseases.* Scarlet fever, septic sore throat, erysipelas, rheumatic fever, rheumatoid arthritis and acute glomerular nephritis are all unknown or at the most exceedingly rare. We have not seen a single instance of any of the above mentioned diseases.

*Maternal and infant mortality.* No accurate vital statistics are as yet available; however, maternal and infant mortality appear to be one of the big health problems of the Marshallese. In the thirty years of Japanese occupancy an effective program for lowering the infant and maternal mortality was never organized. As a consequence approximately 15 per cent of the children are stillborn. Of those alive at birth, approximately 25 per cent are dead at the end of two years. The maternal mortality is more difficult to estimate, but it is close to 10 per cent.

The large number of still births are probably due to a combination of systemic disease of the mother, (chiefly yaws) and to physical injury and

during times of water and food shortage to dehydration and partial starvation.

The infant mortality is due mainly to the "diarrheal diseases", bacillary dysentery and to starvation when, due to food and water shortage, the infant's breast milk supply is curtailed.

The high maternal mortality is due to lack of prenatal care and the handling of deliveries by any woman who happens to be present when accouchement is imminent. On only one island did the native medical practitioner take any interest in obstetrics.

*Clothing.* The missionary inspired clothing adopted by the Marshallese is totally unsuited to this climate. Furthermore the war completely disrupted the supply of cloth and soap to the natives and, as a consequence, those clothes which they have can literally be described as dirty rags. There is no question but that the fungous infections of the skin and the many cases of scabies are due in large measure to this unsuitable clothing. In this connection it is of interest to note that the omnipresent tinea pedis of America is absent among these people who go barefooted.

The sarong type skirt with bare chest for women, and short pants over the well designed native "kal" for men, is the advisable style of clothing.

*Public health.* (1) *Vaccination.* The Japanese had vaccinated the majority of natives who were over five years old. So far as could be determined this was the only type of immunization undertaken.

(2) *Fecal disposal.* Though they tried, the Japanese were not successful in inducing the native to use latrines. Some defecate on the reef at high tide; others hide their feces under a stone or leaf somewhere on the island. (3) *Water supply.* This is either from fresh water rain cisterns or from brackish ground wells. (4) *Flies.* As unused food is scattered on the ground the fly population is large in every village. (5) *Poison snakes.* There are none.

*Native medical practitioners.* Most of the heavily populated atolls have native medical practitioners trained by the Japanese at Jabore. These men have worked for at least four years at the hospital in Jabore. Given supplies and proper supervision, they are capable of administering to the medical needs of the natives.

#### ACKNOWLEDGEMENTS

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# A CONSIDERATION OF THE MECHANISM OF SPLENIC INFARCTS IN MALARIA<sup>1</sup>

R. H. RIGDON

*From the Department of Pathology, School of Medicine, University of Arkansas, Little Rock, Arkansas*

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Taliaferro and Mulligan (1) in 1937 reviewed the literature on malaria and concluded that the areas of necrosis occurring in the spleen have "aroused remarkably little discussion." Bloom and Taliaferro (2) in 1938 apparently were the first to give a detailed consideration to splenic infarction in malaria. These investigators were interested in the etiology and in the regeneration of infarcts in the spleen of canaries infected with *Plasmodium cathemerium*. Bloom and Taliaferro (2) concluded from their studies that "they [infarcts] probably result from the malarial infection, as all attempts to associate them with bacteria, viruses or intravenous injection of india ink have failed."

Hewitt (3) in 1939 studied infarcts in the canary's spleen. Thrombosed vessels were present in every spleen that had an infarct. The occlusions occurred in both the central vein and in its branches. Although infarcts were present as early as the third day following inoculation they were "particularly prevalent on the ninth day" (3). According to Hewitt (3) "it does not seem likely, then, that the production of infarcts is in any way dependent upon the amount of inoculum or upon the number of parasites introduced." If the venous obstructions were due to blockage by large numbers of parasitized cells, "one would not expect to find them early in the disease" (3). Hewitt concluded from his studies that "the great hyperemia which occurs early in infections with this strain of *P. cathemerium* slowed the blood current sufficiently to cause widespread stasis. Thrombosis with resulting infarction, probably occurred due to the extreme blockage of many of the blood channels."

A proliferation of the cells in the wall of some of the large venous sinuses in the spleen was

described in a recent study of the pathological lesions in *M. rhesus* monkeys infected with *P. knowlesi* (4). A proliferation of similar cells was observed also in the wall of the splenic sinuses in young Pekin ducks infected with *P. lophurae* (5). No areas of infarction occurred in the spleen of either these monkeys or the few ducks we studied. The absence of infarcts in the spleen of these monkeys and ducks may be explained by an insufficient degree of proliferation of the reticular-like cells in the wall of the venous sinuses to produce occlusion. The process, therefore, appears to be a variable one.

Hematopoietic tissue is present normally within the lumina of the sinuses during embryonic life in man, from the fourth to the sixth fetal month, and in mammals and lower vertebrates in extra-uterine life (6). Jaffe (7) observed in cases of leukemia that leukemic tissue developed beneath the endothelium of the veins and infrequently in the adventitia of the arteries. Although Jaffe emphasized this pathological process in the spleen in leukemia, apparently he did not associate it with infarction, a lesion that does occur in this disease. Forkner (8) observed that the endothelium in some of the large arteries in a case of monocytic leukemia was separated from the media by an accumulation of monocytes. Ewing (9) has a beautiful illustration of this process of hyperplasia in a large splenic sinus in a case of "pseudo-leukemia." Pinkerston (10) found that the capsule and the trabeculae of the spleen were densely infiltrated by nucleated red cells in a case of acute erythroblastosis. Fried (11) described an extensive infiltration of the wall of the splenic veins by lymphoid cells in a case of leukemia. From these observations it is evident that a varying amount of blood forming tissue may occur in the walls of the large venous sinuses in the normal spleen and in certain diseases.

A proliferation of cells in the wall of the venous sinuses may be associated with the development of splenic infarcts. This relationship was studied in the spleen of a group of human cases infected with *P. falciparum*, monkeys infected with *P. knowlesi*,

<sup>1</sup> The material for the experimental observations and some of the human cases were obtained during the time I was at the University of Tennessee, other material was obtained from the Army Medical Museum through the courtesy of Col. Ash.

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ducks infected with *P. lophurae*, and canaries infected with *P. cathemerium*. Without attempting to review the histology of the spleen in these animals and birds it appears worthwhile to mention briefly a few significant characteristics of this organ in view of their possible relation to infarction.

The capsule and the trabeculae were more pronounced in the spleen of man and monkey than they were in the spleen of the ducks and the canaries used in this study. The canary's spleen had few, if any, trabeculae. The capsule and the walls of the venous sinuses in man and monkey were usually formed by a definite fibrous tissue-like membrane of varying thickness. Collections of cells resembling those in the red pulp were present either between the endothelial cells and the fibrous

those in the splenic pulp were present in the wall of the venous sinuses in each of the ten monkeys (fig. 4). A similar collection of cells was present in the wall of the sinuses in the control monkey.

The cellular reaction in the wall of the venous sinuses in the spleen of ducks has been reported (5). The spleens from ten of these ducks were reviewed. Pulp cells were present in the wall of the venous sinuses of each duck (fig. 5). Hyperplasia of these

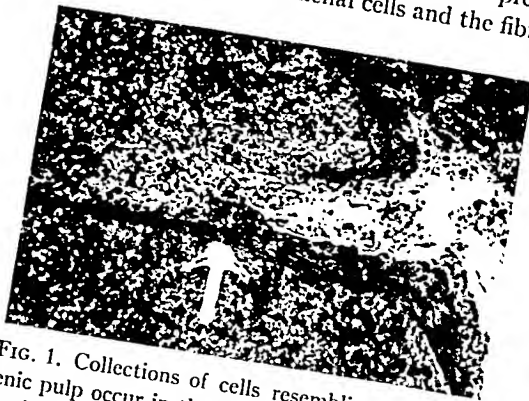


FIG. 1. Collections of cells resembling those in the splenic pulp occur in the wall of this large blood vessel immediately beneath the endothelium. Proliferation of these cells has occurred at the point indicated by the arrow. Spleen from child with *P. falciparum* infection. 96X.

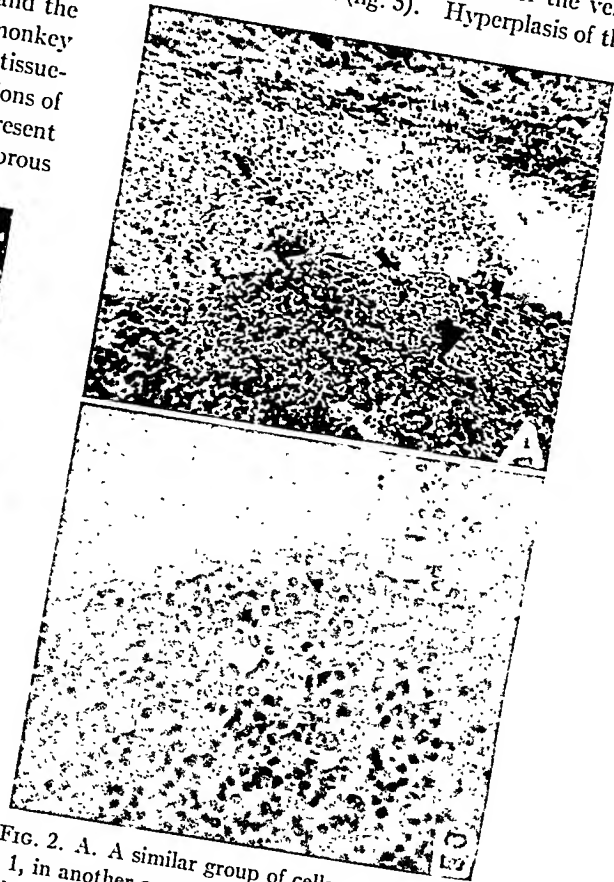


FIG. 2. A. A similar group of cells as shown in figure 1, in another case of *P. falciparum* infection in an adult. X100.  
B. The cells located in the wall of this sinus are identical with those in the pulp. X200.

tissue wall, or they formed the wall of the sinuses (fig. 1 and 2). There was little if any fibrous tissue in the walls of the venous sinuses in the canary's spleen and because of this the sinuses were inconspicuous while those in the duck's spleen were more easily demonstrated (fig. 3). Usually only the central vessels in the canary's spleen had a definite fibrous tissue-like wall. The wall of the venous sinuses in the spleen of the ducks frequently was formed by small amounts of fibrous tissue. It was much less, however, than was present in the spleen of either man or monkey.

Collections of cells were present in the venous sinuses in the monkey's spleen as referred to previously in this paper (4). The spleens were studied from ten monkeys selected at random from a large group infected with *P. knowlesi*. These monkeys either died or were killed during the acute phase of the infection. Cells resembling

cells occurred in the ducks infected for the longer periods. A proliferation of similar cells occurred in the walls of the venous channels in both the liver and the kidney of these birds. The lumen of some of the venous channels were partially obstructed.

The spleens from a group of ten canaries infected with *P. cathemerium* were studied.<sup>2</sup> Serial sections were made on some of these specimens. Complete

<sup>2</sup> Sections on the canaries' spleens were loaned to me by Dr. R. Hewitt for this study.

infarction was present in some of the spleens while others showed only focal areas of necrosis (fig. 6). sinuses. Sometimes the protruding mass of cells contained a pink staining and necrotic-like

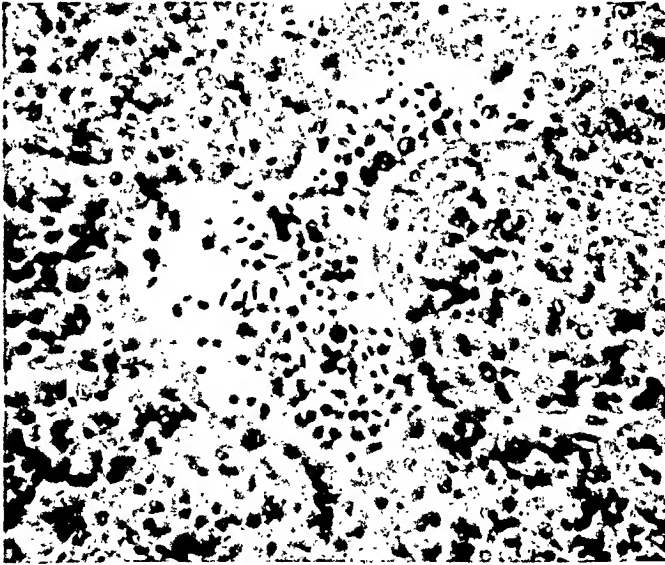


FIG. 3. Cells like those in the splenic pulp frequently form the wall of splenic sinuses in ducks and canaries. Note the absence of any fibrous tissue in the wall. Spleen from Duck infected with *P. lophurac*—Killed on 3rd day. 366X.

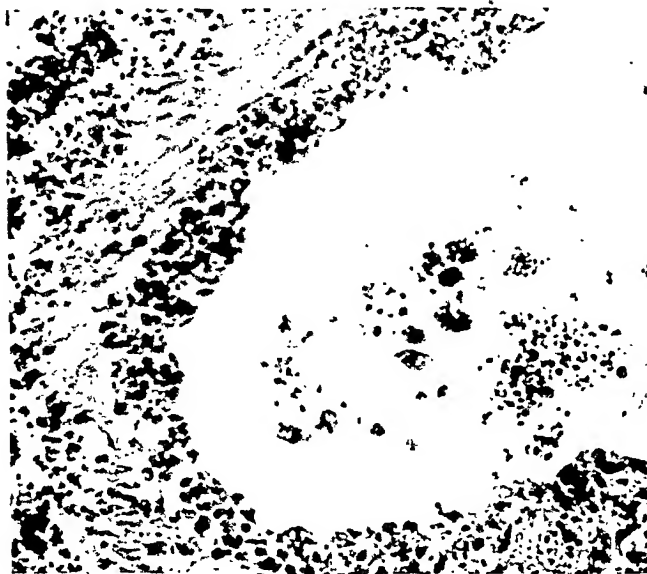


FIG. 4. Cells similar to those in the splenic pulp are frequently found immediately beneath the endothelium in the large blood channels in the monkey's spleen. These cells appear to show definite hyperplastic changes in animals infected with *P. knowlesi*. This animal died 10 days following an intramuscular injection of the parasites. X200.

Vascular occlusions were present in all of these spleens. Groups of cells like those in the splenic pulp protruded into the lumen of many of the venous

material (fig. 6 B). Leucocytes and red blood cells sometimes covered this thrombus-like process. Frequently it was difficult to demonstrate the



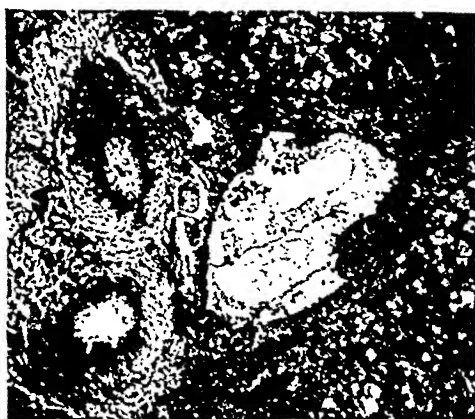


FIG. 5. Large groups of cells like those in the splenic pulp protrude into the lumen of the larger veins. Such a group of cells may be coated with fibrin and circulating blood cells. They may produce obstruction to the blood flow. Duck infected with *P. lephurac*. Killed on 5th day. 96X.

beneath the endothelium of the central artery (fig. 7).

Collections of reticular-like cells were present beneath the endothelium in the large venous sinuses in the spleen of humans infected with malaria. The size of these groups of cells varied. One of the spleens we studied showed numerous focal areas of necrosis in the pulp and collections of reticular-like cells were present in the wall of the splenic vessels. The association of the hyperplasia of the cells in the vessels and the focal areas of necrosis in this case may be significant in the development of splenic infarcts.

#### DISCUSSION

Many investigators have described collections of cells in the wall of the larger sinuses in the spleen. The consensus appears to be that these cells may be considered as part of the blood forming group.



FIG. 6. A—Infarct in a canary's spleen. Necrosis is shown on the right side of the picture. The lumen of the two vessels at the point of the arrow is completely occluded by a fresh thrombus-like process. The vessel at X is shown in Figure B. There is a thrombus-like mass attached to this vessel wall. The bird was infected with *P. cathemerium*. A, 18X; B, 96X.

lumen of the smaller sinuses in the canary's spleen since the cells in the wall were continuous with those in the splenic pulp. Sometimes collections of these pulp cells were present immediately

It is not surprising to find hyperplasia of these cells in the wall of the venous sinuses in malaria since splenomegaly apparently always occurs in this disease. The degree of hyperplasia of these

cells may vary in the different species and also in the different vessels of the same spleen. It is suggested that this hyperplasia results from the anemia. It is evident, therefore, that hyperplasia of these cells may occur in diseases other than malaria 8-10. Normally in the wall of the venous sinuses in the spleen of man, monkey, duck, and canary there are cells similar to those in the red pulp. Sometimes these cells are located between the endothelium and the vessel wall. At other times they are continuous with the cells in the red pulp. It is well known that the histological structure and the type of circulation varies in different species (12).

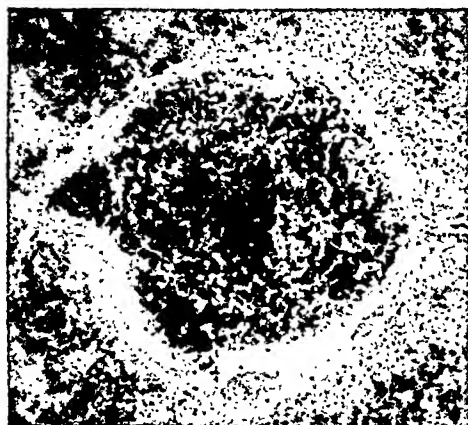


FIG. 7. Groups of cells are present in the wall of the central artery in the canary's spleen. These cells resemble those in the splenic pulp and apparently proliferate during malarial infection.  $\times 96$ .

There is a slowing of the circulation produced by these hyperplastic cells projecting into the lumen of the venous sinuses. As a result of this decrease in the rate of flow, leucocytes may accumulate on the periphery of these clusters of reticular-like cells. A thrombus may develop and ultimately completely occlude the lumen. This process occurs most frequently in the spleen of canaries infected with *P. cathemerium*. Bloom and Taliaferro (2) have observed an infiltration of non-granular heterophil leucocytes into the walls of the veins during the early period of infection in a few canaries. Some of these birds showed either partial or complete thrombosis of the splenic veins.

A thrombotic occlusion apparently is not the only process that affects the circulation in the acute types of malarial infection. The blood current may be slowed as a result of the anemia, and anoxia of the cardiac muscle may occur. Associated with

anoxia may be an increase in capillary permeability. This, likewise, may affect the general circulation. It would appear, therefore, that anemia and a failing myocardium may contribute to the local development of vascular occlusions which have their beginning in the hyperplasia of the reticular cells in the wall of the venous sinuses. Osler (13) and Hewitt (3) both referred to the sluggish circulation in malaria. Rigdon and Stratman-Thomas (4) discussed its relationship to the pathological lesions occurring in monkeys infected with *P. knowlesi*.

Splenic infarcts have been observed in man infected with *P. falciparum*, and in monkeys infected with *P. knowlesi* (14). The focal areas of necrosis in the spleen of one of our human cases resembled those present in the spleen of some of the canaries. Infarcts did not occur in the spleen of either the human cases or in the monkeys used in this study. The frequency in which infarcts occur may be influenced by the type of the infection and the duration of the disease. Hewitt (3) observed infarcts in the spleen of 47% of the canaries infected with *P. cathemerium*. Infarction in the canary's spleen occurs more often on the 9th day of the infection (3). The degree of hyperplasia of the spleen in ducks progresses during the first several days of the disease. It appears, therefore, that the hyperplasia of the cells in the wall of the veins is a part of the general process of hyperplasia of the splenic pulp. It is suggested that this process is nonspecific in the spleen in malaria, since a similar lesion occurs in the liver and in the kidneys of the ducks (5). If hyperplasia of the reticular cells in the wall of the veins progress, either partial or complete occlusion may result with subsequent infarction. This seems to be a reasonable explanation for the development of splenic infarcts, as ascertained from a review of the literature and also from experimental studies. This opinion of the etiology of infarcts apparently is supported by the fact that Bloom and Taliaferro (2) were unable to produce infarcts in the canary's spleen by an intravenous injection of india ink. Hewitt (3) could not associate infarcts with the number of parasites injected. He also observed infarcts in the canary's spleen more frequently after the ninth day of the disease. This interval likewise coincides with that of the time of development of hyperplasia. Variations in the frequency of splenic infarcts may be influenced by the normal histological structure of the spleen, by the type of circulation, and also by the type of malarial infection.

## SUMMARY

It is suggested that the infarcts occurring in the spleen in malaria may result from an obstruction produced by hyperplasia of the reticular-like cells located within the walls of the venous sinuses. Leucocytes and red blood cells may adhere to the surface of these masses of reticular-like cells since the circulation in malaria is slowed. The normal histological structures in the spleen of different animals are discussed that may predispose to vascular obstruction.

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# A CHECK LIST OF THE MITE VECTORS AND ANIMAL RESERVOIRS OF TSUTSUGAMUSHI DISEASE<sup>1</sup>

ROGER W. WILLIAMS

*The DeLamar Institute of Public Health, College of Physicians and Surgeons, Columbia University*

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Tsutsugamushi disease, an ancient malady known since the sixth century, is today presenting a problem, among our military personnel, which is taking on considerable significance. Major Ahlm and Captain Lipshutz (14) made the following statements in reference to this ailment: "Until recently the condition had not been given the space it deserves in medical textbooks, owing in part to our lack of interest from the economic standpoint in many of the tropical and subtropical countries. The advent of World War II has, however, changed the picture entirely. We now find not only men of the allied armed forces but our own men suffering the ravages of this fever in many tropical areas. The total man days lost from this disease have presented a problem. This together with the great advance in air transportation expected in the post war period tends to make the disease one of considerable military and economic importance."

Tsutsugamushi disease has many synonyms, and in recent decades ailments which appear to be identical with it clinically and etiologically have been described under other names in various localities. Some of these synonyms are: Tsutsugamushibyō, Shimamushi, Japanese River fever, Flood fever, Kedani fever, Mite fever, Scrub typhus (K form), Rural typhus, Tropical typhus, Pseudo-typhus, Mossman Fever and until recently the so-called Coastal Fever of North Queensland Australia. Haeslip (15) has shown that the latter malady is caused by a bacillus (*B. tropica*) and that it is probably transmitted by the same vectors as Tsutsugamushi disease.

The geographical boundries of Tsutsugamushi

disease are as yet probably very incompletely determined. It has been reported from Japan, China, Formosa, Pescadores Islands, Philippine Islands, Indo-China, Cambodia, Malay Peninsula, Sumatra, Java, Borneo, New Guinea, North Queensland Australia, Bako (?) Islands, India, Ceylon, and Samoa. Table 1 includes only localities where studies have been made of vectors and reservoir animals.

The etiological agent is identified as a rickettsial form known as *Rickettsia nipponica*, but it has been named by various authors, *R. tsutsugamushi*, *R. orientalis*, and *R. akamushi*.

The infection which is not communicable naturally from person to person, is transmitted to man from certain rodents and marsupials, for the most part through the bite of various species of larval mites of the genus *Trombicula*. The larvae of these Acarids feed but once during this stage, and this is the only parasitic stage during the life of the mites. Therefore, the rickettsiae must necessarily be carried through the various stages of development and transmitted through the egg to the next generation of mites, and according to Hayashi and Kato (16), to a number of subsequent generations.

There is some evidence which indicates that perhaps mites are not the only vectors. In several localities ticks have been suspected. Van der Schroeffer (17) studied cases in north Sumatra which appeared to be transmitted by members of the tick genus *Amblyomma*. A report prepared in the Office of the U. S. American Typhus Commission (18) would seem to indicate that in New Guinea at least, the itch mite may not always be involved, ". . . since little correlation, if any, was found between the incidence of 'scrub itch' and the frequency of infection with scrub typhus. In some areas there was considerable complaint of 'scrub-itch' but no scrub typhus, while in other areas there was no complaint of 'scrub-itch' but there were cases of scrub typhus." Although mites may not be the only vectors of Tsutsugamushi

<sup>1</sup> The compiling of this information was a result of a comprehensive bibliography on chigger mites (#13) compiled while engaged on chigger research during the summer and winter of 1942 at the School of Public Health, University of North Carolina, Chapel Hill, North Carolina. The research was financed by the Eli Lilly Company through Dr. H. W. Brown to whom I am indebted for his helpful suggestions.

disease they appear to be by far the most important known at the present time.

when the malady is not present it may be that the mites can be found deep in the ears and on the

TABLE 1  
*Mite vectors and reservoir animals of Tsutsugamushi disease*

GEOGRAPHICAL DISTRIBUTION	MITE VECTOR	RESERVOIR
Australia	<i>Trombicula deliensis</i> Walsh, 1923 (1) <i>T. minor</i> (Hirst) Sambon, 1927 (1) † <i>Laelaps australiensis</i> (2)	* <i>Isodon torosus</i> —Bandicoot (1) * <i>Melomys littoralis</i> —Rat (1) * <i>Rattus assimilis</i> —Rat * <i>Rattus conatus</i> * <i>Rattus norvegicus</i> (white strain) (1) * <i>Rattus norvegicus</i> (black & white strain) (1) * <i>Rattus rattus</i> (1)
China	<i>T. akamushi</i> Brumpt, 1910 (3)	? ? ? ? ?
Formosa	<i>T. akamushi</i> (3, 4)	<i>Rattus rattus rufescens</i> (3, 9) <i>Rattus rattus rattus</i> (5) * <i>Rattus losea</i> (5, 11) <i>Rattus</i> ( <i>Apodemus</i> ) <i>agrarius</i> (5, 3) <i>Rattus norvegicus</i> (3) <i>Rattus musculus</i> (3) <i>Pachyura murina</i> —Musk Shrew (3)
India	<i>T. deliensis</i> (6) <i>T. acuticellaris</i> Walsh, 1923 (6)	<i>Mus</i> ( <i>Rattus</i> ) <i>rattus</i> (6) <i>Mus decumanus</i> <i>Mus bactrianus</i> (6) <i>Golunda ellioti</i> —Indian brush rat (6) <i>Nesocia begalensis</i> —Mole rat (6)
Japan	<i>T. akamushi</i> (4, 7, 9)	* <i>Microtus montebelli</i> —Vole (4, 7, 9) <i>Arvicola hatanedzumii</i> —Field mouse (8)
Malaya	<i>T. akamushi</i> (4) <i>T. deliensis</i> (9) ‡ <i>Schöngastia shüffneri</i> Walsh, 1923 (9)	<i>Mus rattus</i> (4) <i>Mus concolor</i> (4)
New Guinea	§ <i>T. minor</i> (10) <i>T. deliensis</i> (1)	<i>Echymipera cockerelli</i> —Bandicoot (10)
Pescadores Is.	<i>T. akamushi</i> (11)	* <i>Mus rattus rufescens</i> (11)
Sumatra	<i>T. akamushi</i> (12) <i>T. deliensis</i> (9, 3) <i>S. shüffneri</i> (9, 3)	<i>Rattus rattus diardii</i> (9, 3) <i>Mus concolor</i> (3)

\* Animals in which the rickettsial organism has been demonstrated in nature, and which act as hosts of the mite vectors.

† Not a chigger mite.

‡ Thought to transmit the disease only from man to man. Belongs to genus indicated rather than *Neoschöngastia* as placed by some authors.

§ Some authors have placed the New Guinea form of this mite in the genus *Nesochöngastia*.

It is quite possible that these mite vectors may be collected the year around even in those areas where the disease is seasonal. During the seasons

eyelids of their rodent hosts. Japanese workers have found them in the winter months in such locations on bank voles. *Trombicula autumnalis*

Shaw, a closely related species, passes the winter in the ears of rodents in England and I have found the common North American chigger, *Eutrombicula alfreddugesi* (Oudemans) in the ears and on the eyelids of rabbits and squirrels in December and January (Williams (19)).

Frequently the statement is made that the animal reservoir is believed to be a specific rodent or rodents. In many cases the actual reservoir in nature has been demonstrated so that it is no longer a question of merely believing or supposing, for at least ten species and strains are now known to be natural reservoirs of *R. tsutsugamushi*. This list includes six species of rat and one species of bandicoot from Australia, one rat species from Formosa, one species of rat from the Pescadores Islands and one species of vole from Japan.

There may be many animals which serve as reservoirs whose role in the story of this disease has as yet not been discovered, since the mite vectors also attach and feed on dogs, cats, fowl, buffalo, monkeys, lizards, particularly wild birds, and no doubt many other animals. Wild birds do at least serve as distributing agents of the mites. According to Walsh (20) the mite larvae have been found on *Acrocephalus orientalis* a bird which migrates southward from Japan in the winter and may possibly carry the disease to distant countries. Keukenschrijver (10) examined a number of crow-pheasants (*Centropus javanicus*) which frequent the undergrowth and jungle grass in Malaya, with the result that he found the majority of them infected with *Trombicula deliensis*.

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# PROBABLE ROLE OF THE CAT FLEA, CTENOCEPHALIDES FELIS, IN TRANSMISSION OF MURINE TYPHUS

J. V. IRONS, S. W. BOHLS, D. C. THURMAN, JR.,<sup>1</sup> AND T. MCGREGOR<sup>2</sup>

From the State Health Department Laboratories, Austin, Texas

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Since the recovery of a strain of typhus from rat fleas by Dyer, Rumreich, and Badger (1) and from the brain of a wild rat by Mooser, Castaneda, and Zinsser (2) in 1931, the importance of the rat and the oriental rat flea, *Xenopsylla cheopis*, in the transmission of murine typhus has become well established. Sparrow (3) and others have reported finding typhus rickettsiae in house mice, and Brigham (4) obtained a typhus strain from a field mouse; occasional observations have been reported which suggest that additional murine or other animals may serve as hosts, and many animals have been experimentally infected. Thus, Lepine and Lorando (5) obtained experimental infections in cats which were usually of the inapparent type; this finding was soon confirmed by Brigham and Dyer (6). Similarly, several species of fleas in addition to *Xenopsylla cheopis* have been experimentally infected and found capable of transmitting infection. Liu and Zia (7) isolated typhus rickettsiae not only from mouse fleas but also from mice during a household outbreak of typhus, and Brigham (8) isolated typhus rickettsiae from naturally infected *Echidnophaga gallinacea*, the chicken flea, taken from the rat.

Isolation of a strain of murine typhus from the cat flea, *Ctenocephalides felis*, and some observations suggesting transmission of the infection to man through the agency of these fleas harbored by kittens provide the basis for the foregoing report.

In the fall of 1942 we visited the J. family of Austin, Texas, all of whom had typhus with onset during November 1-10, 1942. In each instance the clinical diagnosis had been confirmed by the Weil-Felix test. Rats or mice had not been troublesome, and there was very little evidence of the presence of rodents on the premises. Mrs. J. was insistent that their infections had been acquired from a kitten which had been picked up at a local feed store about ten days before onset of

her daughter's illness about November 1st. Two or three days later Mrs. J. had her onset, shortly followed by onset of her husband's illness. When Mr. J. became ill, the kitten was destroyed, but previously it had been permitted the run of the house. No attempts had been made to rid the kitten of fleas.

At the feed store whence the kitten in question had been obtained, we were able to locate the two litter mates, from which approximately two hundred fleas were obtained; these fleas were subsequently identified by us as *Ctenocephalides felis* without exception. A small group of these fleas was sent to the U. S. P. H. S. Laboratory, Hamilton, Montana, for identification; these fleas were also identified as *Ctenocephalides felis*. Meanwhile, fleas were grouped in three pools of fifty-five each and maintained in the frozen state until saline suspensions were prepared for inoculation of rats and male guinea pigs; a pool of flea eggs was also inoculated into a rat. All inoculations were made intraperitoneally, in the usual manner for detection of murine typhus.

From table 1, it is seen that with pools #2 and #3, following an incubation period of a week to ten days, guinea pigs became febrile and developed scrotal reactions consistent with experimental murine typhus. Negative results were obtained with flea pool #1. Fourteen days after inoculation of rats with each of the four pools, these animals were sacrificed, brains were removed, and heavy saline suspensions of individual brains were inoculated into male guinea pigs in the usual manner. Subsequently, Neill-Mooser reactions consisting of elevated temperature and scrotal involvement of guinea pigs were obtained, with reference to rats inoculated with flea pools #2 or #3; again, a negative result was obtained relative to flea pool #1, as with the pool of flea eggs. The incubation periods in guinea pigs inoculated with rat brain suspensions were shortened as compared with guinea pigs inoculated with flea suspensions. The characteristic infection was

<sup>1</sup> Now Assistant Sanitarian, U. S. P. H. S., Atlanta, Georgia.

<sup>2</sup> Now 1st Lt., Sanitary Corps, U. S. Army.



readily transmissible in guinea pigs. Incubation periods on guinea pigs with blood inoculations were longer and more variable than with testicular tunica inoculations. Temperatures of infected guinea pigs seldom exceeded 104.5°F. and gradually reached normal levels in a few days; the mortality was practically nil. With animals sacrificed early and at the height of the febrile course, the scrotal sac was swollen and inflamed and testicles were not palpable; as a rule, both testicular tunics were found to be thickened, hemorrhagic, and adherent, but on occasion the reaction was unilateral. Generally, there was no evidence of infection at the site of inoculation and little or no evidence of peritonitis. Masses of

Unlike the J. family, Mrs. Y. attempted to rid her kittens of fleas with the result that "fleas were all over the house for days"; Mrs. Y. also asserted she was bitten repeatedly by these fleas.

We were unable to relate other cases of typhus in recent years to the particular feed store in question. Careful questioning indicated, however, that some of the employees in years past had been ill with symptoms suggestive of typhus, and it was believed that the illness had been acquired "somehow or other from eating on the hay bales or sleeping on the hay bales after lunch". Rats had long been troublesome but poisoning efforts had been discouraging, it developed, because the "cats rather than the rats ate the poison".

TABLE 1  
*Results of inoculation of rats and male guinea pigs with pools of cat fleas*

LOT NO.	NO. OF FLEAS	GUINEA PIG NO.	RESULTS	RAT	GUINEA PIG NO.	RESULTS
1	55	690	Neg.	R1†	702	Neg.
2	55	691	Pos. (8)*	R2	703	Pos. (6)
3	55	692	Pos. (10)	R3	705	Pos. (6)
4	Many eggs			R4	704	Neg.

\* Days required for scrotal reaction and temperature elevation.

† On 14th day each rat brain suspension was inoculated into a male guinea pig.

minute, rod-like organisms characteristic of murine typhus rickettsiae were demonstrable in cells from the testicular tunica. Bacteriological cultures failed to reveal the presence of any significant organism and *Salmonella* organisms particularly were not encountered. These findings were entirely consistent with our experience with several experimental murine typhus strains obtained from human blood, rat brain tissues, or suspensions of fleas. Two lines of transfer were carried through four and six passages before termination of the investigation, and served clearly to identify the infective agent as that of typhus.

Blood samples from the kittens in question reacted negatively in the Weil-Felix test. Suspensions of kitten brains and spleens were inoculated into male guinea pigs, but none showed elevated temperature or scrotal reaction during a twenty-one day period of observation.

During the course of these experiments, our attention was directed to Mrs. Y., a recent typhus convalescent. A few days previous to onset of her illness, Mrs. Y. had obtained two large kittens from the feed store in question. She selected the two larger in preference to the three smaller kittens.

#### *Typhus infection in a laboratory worker apparently acquired from cat fleas*

On or about December 3rd, 1942, Bacteriologist, D. A. K., who had assisted with the conduct of these investigations, developed a slight fever and headache, without evidence of respiratory tract infection, but continued at work until December 7 when she first mentioned her illness. Some data on her infection are given in table 2, and it seems quite probable that D. A. K. had acquired infection as a result of contact with the infected fleas with which she had worked. Infection was unusually mild, possibly because of previous vaccinations.

#### *Attempted infection of kittens and fleas with guinea pig passaged strain of murine typhus*

In May, 1943, a mother cat with very young kittens and an abundance of fleas were obtained. A pool of fleas, each of which was identified as *Ctenocephalides felis*, was crushed and a saline suspension was inoculated into two male guinea pigs, with subsequently negative results. Subsequently two kittens were inoculated intraperitoneally with a fresh guinea pig tunica typhus

# CAT FLEA IN TRANSMISSION OF MURINE TYPHUS

suspension. The strain employed was originally started from the brain of a local rat and was subsequently passaged four times in guinea pigs. The dose employed was sufficient to produce a typical Neill-Mooser reaction in the control guinea pig on the seventh day. The kittens and fleas were maintained in a room where the temperature varied from 70° to 85°F., and in strict isolation for fourteen days, when pools of fleas were obtained and suspensions were inoculated into male guinea pigs in the usual manner. On the fourteenth day the kittens also were sacrificed, and suspensions of their brains and spleens were tested by guinea pig inoculation; the kittens had remained afebrile. Completely negative results were obtained both with the kitten tissues and fleas suspensions.

inclined to suspect that the cat fleas acquired the infective agent from the kittens.

These findings are illustrative of the complex biology of endemic typhus rickettsiae but in no way detract from the fundamental importance of the rat and its fleas in the epidemiology of murine typhus. In endemic areas feed stores are notorious as typhus foci *par excellence*. A typhus strain was obtained from a rat in the neighborhood of this feed store. This apparent implication of cat fleas in transmission of murine typhus perhaps was an exceptional occurrence. It is noteworthy that all members of two households including the only child became infected. These cases also included nearly all cases of typhus seen by physicians in Austin, Texas in 1942.

TABLE 2  
Data on case of D. A. K.\*

DATE OF PROBABLE EXPOSURE	NATURE OF PROBABLE EXPOSURE	DATE OF ONSET	COURSE OF INFECTION	GUINEA PIG INOCULATIONS DEC. 10TH	RESULT OF WEIL-FELIX TESTS
November 20, 21, or 22, 1942	Mashed a stray cat flea, believed a flea bit her.	About Dec. 3rd; worked until Dec. 7th.	No spots found; last day of fever—Dec. 12th; returned to work rather "rundown," Dec. 15th.	Negative	Dec. 8th—negative; Dec. 15th — positive 1:320

\* Received subcutaneously a total of 2.5 cc. vaccine (Castaneda) in three doses a week apart in summer of 1939 and again fresh Lilly vaccine (Castaneda type) given in similar doses in December, 1941.

## DISCUSSION

So far as we are aware, this is the first report of recovery of murine typhus from naturally infected *Ctenocephalides felis*, the cat flea. The investigation was prompted by a suspicion that fleas on kittens carried home from a feed store were responsible for four cases of typhus fever. During the laboratory investigation, a bacteriologist assisting in the work acquired typhus, evidently as result of contact with infected cat fleas or tissue suspensions of these fleas.

Failure to produce typhus with one of the three flea pools suggests that relatively few of the fleas harbored the causative rickettsiae. Since we failed to find evidence of past or present infection in the kittens, it might be postulated that the fleas in question had acquired the infective agent from rats. It is well known that both the cat and the dog flea will attack other animals, including man, although we have never found either flea on rats and Prince (9) found only three cat fleas in an examination of 4,188 rats in the western United States. We are

## SUMMARY

Typical murine typhus was obtained in male guinea pigs by inoculation of suspensions of *Ctenocephalides felis*, the cat flea. Negative findings were obtained with a suspension of flea eggs.

At least five cases of typhus including one laboratory infection probably were acquired through the agency of cat fleas harbored by kittens carried away from a feed store.

## RESUMEN

El Tifus típico murino fué obtenido en un cuilo o cuyo macho por inoculación de suspensiones de la pulga de gatos (*Ctenocephalides felis*). Resultados negativos se obtuvieron con la suspensión de huevos de las pulgas.

Por lo menos cinco casos de tifus incluyendo una infección de Laboratorio, probablemente fueron adquiridas por intermedio de las pulgas albergadas por dos gatitos que las esparcieron desde una Tienda de alimentos.

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# SPONTANEOUS HISTOPLASMOSIS OCCURRING IN A DOG<sup>1</sup>

WILLIAM P. CALLAHAN, JR., M.D.

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

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In 1939 De Monbreun reported an example of generalized infection in a dog by *Histoplasma capsulatum*. A diagnosis was made on a biopsy specimen from the liver, and cultures of the peripheral blood before death yielded the mycelial form of the organism. Cultures from the other viscera at autopsy also showed the presence of *Histoplasma*, and the infection was transmitted to other dogs parenterally and by mouth. This was the first proven case of naturally occurring histoplasmosis in animals although spontaneous instances of the disease had been previously observed by the examination of tissue sections from other species of animals. Sangiorgi (1922) described parasites in the viscera of mice which he thought to be blastomycosis but were considered by Wenyon (1926) to be suggestive of *Cryptococcus* or *Histoplasma*. Shortt (1923) described a spontaneous infection in laboratory mice with *Cryptococcus muris* although a similarity to *Histoplasma* was noted. Levine, Dunlap and Graham (1938) reported an intracellular parasite in the liver, spleen and lungs of a ferret which they thought was similar in appearance to *Cryptococcus*. This was considered by Meloney (1940) to be suggestive of histoplasmosis from the description and the photomicrographs published. Summerhill (1941) identified intracellular parasites which he considered to be *Histoplasma* in the mesenteric lymph nodes and the lungs of a cat. More recently Thuringer (1944) reported the second instance of spontaneous infection in a dog by *Histoplasma capsulatum*.

In his report De Monbreun suggests the possibility that the dog may serve as a natural host and that the disease might possibly be transmitted to man in infected excreta and secretions, or that the flea or tick may act as a transmitting agent. In his case and also in the case to be reported, the dogs belonged to physicians, who performed or had

autopsies performed, and the organs were sectioned for microscopic study. In this manner the diagnosis was made and in the first instance substantiated by cultures and the reproduction of the disease in other animals. Also in the case reported by Thuringer the diagnosis was made at autopsy.

Since 1939 many cases of human histoplasmosis have been added to the literature showing that this disease is much more prevalent than was previously supposed. Therefore, it is felt that an additional instance of spontaneous infection in a dog should be reported.

## REPORT OF CASE

The dog was a seven year old female Springer Spaniel, belonging to a St. Louis physician. It was born and raised in this community and appeared perfectly well until August, 1943. At this time it was taken to a kennel in the city to be boarded while the family was on vacation. In the kennel it was placed in a runway by itself although there were several dogs in adjacent houses. The animal was boarded here for four weeks and was then returned home where it began to refuse food and passed several large, black stools a day. It became progressively weaker and died after an illness of two months.

## AUTOPSY FINDINGS

The body was emaciated and there was an icteric tint to the sclera. The peritoneal cavity contained approximately 200 cubic centimeters of slightly blood tinged fluid, and there was a sero-fibrinous exudate on the visceral and parietal peritoneum. The mesenteric, peripancreatic and periportal lymph nodes were enlarged and firm. On cut section they were grayish-white with numerous small yellow foci scattered throughout the substance. The spleen was enlarged to about three times normal size and on cut section was a dark red color. The Malpighian corpuscles were obscured and no areas of necrosis were seen. No gross pathologic changes were observed in the heart,

<sup>1</sup>The author is indebted to Dr. Oscar C. Zink, to whom the dog belonged, and to Dr. L. S. Walsh, who performed the autopsy, for their permission to make this study.

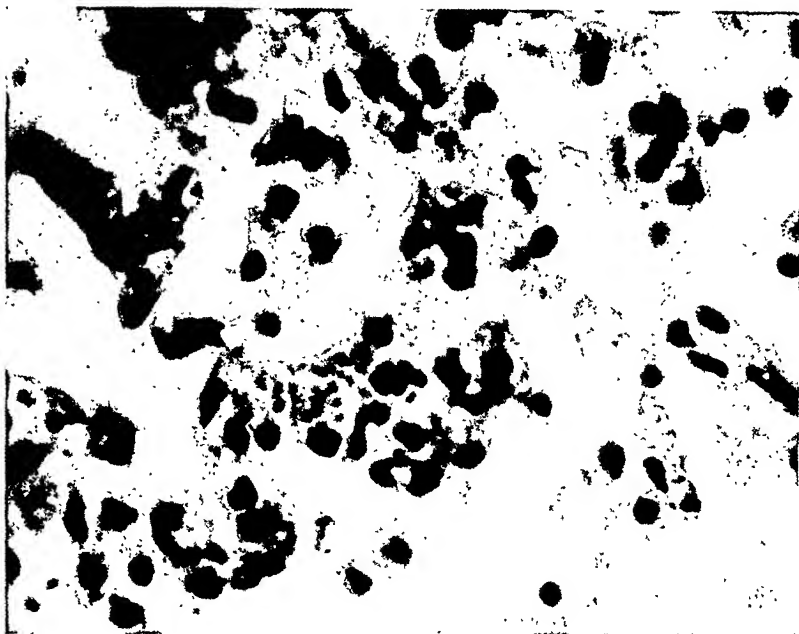


FIG. 1. ORGANISMS WITHIN THE RETICULO-ENDOTHELIAL CELLS LINING THE SPLENIC SINUSOIDS. HEMATOXYLIN-EOSIN.  $\times 570$

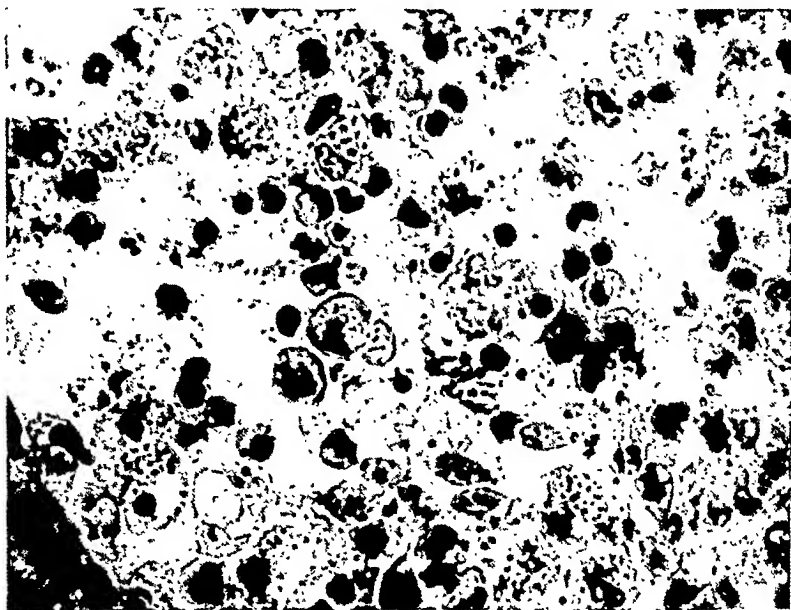


FIG. 2. ORGANISMS WITHIN LARGE MACROPHAGES AND RETICULO-ENDOTHELIAL CELLS IN A MESENTERIC LYMPH NODE. HEMATOXYLIN-EOSIN.  $\times 570$

lungs, pancreas, adrenals, kidneys, and the gastrointestinal tract. The tracheobronchial and bronchopulmonary lymph nodes were moderately enlarged and firm. No cultures were taken and no direct smears were made at the time of autopsy.

#### MICROSCOPIC EXAMINATION

When the author saw the case, three months after the dog had died, microscopic sections of the spleen and mesenteric lymph nodes were the only ones available.

### *Spleen*

The normal architecture of the spleen was greatly distorted. The splenic follicles were small and few in number, and in the red pulp were numerous large macrophages and plasma cells. There was a marked proliferation of the reticulo-endothelial cells within which there were numerous small organisms which were oval in shape with a thin non-staining membrane surrounding a hyperchromatic mass which appeared as a small dot. The nucleus was round or oval in most instances, but occasionally one was seen which was crescentic. The cells contained up to 15-20 organisms, but in most instances 4-8 were present. Organisms were also found in the reticulo-endothelial cells lining the sinusoids and surrounding the small arterioles within the splenic follicles. Many of the large macrophages contained vacuoles and a small amount of brown pigment. A moderate number of multinucleated cells were present and a few foci of extramedullary hemopoiesis. Numerous small lymphocytes were present, irregularly dispersed throughout the splenic substance.

### *Mesenteric lymph node*

Within the mesenteric lymph node the cells were separated and the small venules, capillaries and lymphatics were greatly dilated. Throughout the substance of the node and also in the peripheral sinus a small amount of precipitated edema fluid was present. There was marked proliferation of the reticulo-endothelial cells throughout the lymph node and in many of these large endothelial cells organisms morphologically identical with those seen in the spleen were found. The germinal centers were decreased in number, and there was infiltration with large macrophages, plasma cells and a few scattered small lymphocytes. There were large foci of necrosis which appeared as amorphous, granular eosinophilic material. An occasional multinucleated cell was seen near the periphery of the node. Throughout the entire section numerous small foci of hemorrhage were observed.

### DISCUSSION

Although sections of only two organs were available for microscopic study, organisms identifiable as *Histoplasma capsulatum* were found in large numbers. The lesions in the mesenteric lymph nodes showed large foci of necrosis while those in the spleen consisted chiefly of reticulo-

endothelial hyperplasia and parasitism of these cells by *Histoplasma*. The difference in the reaction of these two organs might suggest that the organisms were present in the lymph nodes earlier in the disease than they were in the spleen. Although no direct evidence is available in this case it is possible that the lymph nodes were involved at an early stage and that after destruction took place large numbers of organisms may have been liberated to be carried to other parts of the body. From the type of reaction seen in other instances of histoplasmosis the lymph nodes form the first line of defense.

The occurrence of only seventy-seven instances of histoplasmosis throughout the world (Parsons) suggests that man to man transmission is unlikely and that there must be some outside reservoir of the disease. Beamer, Smith and Barnett have shown the organism is resistant to drying, even for long periods of time, and it is possible that the fungus may be harbored in the soil or be present on vegetable matter and that air-borne infection may occur. The frequency of involvement of the lungs as well as several instances of lesions in the trachea and larynx suggests the respiratory tract as the route of infection. However, Henderson, Pinkerton and Moore failed to infect guinea pigs and dogs by intratracheal injections of cultures of the organism. The presence of lesions in the gastro-intestinal tract in several instances in man as well as in experimentally infected animals supports the postulation that this may be a route of infection. De Monbreun has produced the disease in dogs by feeding cultures with their food, but Beamer and his associates were unable to transmit the disease to guinea pigs in this manner. The lesions in the intestines suggest that transmission would be possible through infected excreta. Three instances of the disease in man from St. Louis would lend support to the above mentioned portals of entry. In one the first lesion to appear was a small indurated ulcer on the tongue of a 71 year old man which grossly simulated carcinoma to the extent that it was treated with radiant energy. Microscopic examination of a biopsy from the ulcer revealed numerous intracellular organisms identifiable as *Histoplasma capsulatum*. The patient died several months later, but no autopsy was performed. In the other two instances symptoms of respiratory difficulty were the first to appear and endoscopic examination revealed partial obstruction of the air passages by a small

mass. Microscopic examination of biopsies from these areas showed numerous organisms morphologically identical with *Histoplasma capsulatum*. One of these patients later died of a generalized infection and the other is still alive three months after the diagnosis was made. Whether or not these lesions represent the primary site of the infection is questionable, but clinically they were the first to appear. Moore and Jorstad have collected 19 cases whose presenting symptoms were referable to the mouth, nose or throat and the frequency of early involvement of these parts suggests either the respiratory or gastro-intestinal tracts as the portal of entry. Also Henderson and his associates have emphasized the frequency of involvement of the mesenteric lymph nodes and point out, that, as De Monbreun demonstrated, organisms could apparently pass through the intestinal mucosa to the mesenteric lymph nodes without causing demonstrable lesions. The possibility of insect vectors must be considered as some are known to harbor intracellular yeasts. In a case reported by German and his associates a black ant was seen on an infant shortly before the appearance of a generalized infection with *Histoplasma* which terminated in death. Also skin lesions were observed by Beamer, Smith and Barnett but no microscopic examination was made so it is unknown whether or not parasites were present in the cutaneous eruption. As De Monbreun pointed out, it is possible that the tick or flea may serve as a transmitting agent since the fungus has been demonstrated in the blood stream of experimentally infected dogs, and has also been observed in the peripheral blood of patients suffering from the disease.

The occurrence of spontaneous histoplasmosis in three dogs and a cat is of considerable importance in attempting to establish a source of human infection. Since a number of cases have been reported from the St. Louis area (Beamer), it is of importance to recognize this disease as occurring here also in animal reservoirs, since a large number of these cases have been in children who are in close contact with animal pets. Although no definite correlation between the disease in animals and man can be established, it is postulated that

its simultaneous occurrence in man and in animal pets may be of considerable significance.

#### SUMMARY AND CONCLUSIONS

1. The third instance of a generalized infection in a dog by *Histoplasma capsulatum* is reported.
2. Material was not available for the cultivation or passage to other animals, but the morphologic characteristics and the reactions of the host are identical with previously described cases of this disease.
3. The importance of the occurrence of the disease in an animal pet in an area in which a number of human cases have been reported is stressed.
4. The possibility of the various methods of transmission from dog to man as postulated by De Monbreun is discussed.
5. The majority of evidence suggests either the respiratory or the gastro-intestinal tracts as the portal of entry.

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# COMPARATIVE AMEBACIDAL ACTIVITY OF PHENYL ARSINE OXIDE (MAPHARSEN), RELATED ARSENICALS AND OTHER AGENTS<sup>1</sup>

HAMILTON H. ANDERSON, AND THOMAS T. K. CHUAN<sup>2</sup>

From the Department of Pharmacology, Peiping Union Medical College, Peking, China, and the Division of Pharmacology, University of California Medical School, San Francisco

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There are many reports on the use of the hemialcoholate of 3-amino-4-hydroxyphenylarsine oxide hydrochloride (mapharsen) in the treatment of experimental syphilitic animals in comparison with the arsphenamines and this drug is used clinically with considerable success. The first study by Tatum and Cooper (1) in 1932 indicated that the therapeutic index in experimental trypanosomiasis was relatively high as compared with other effective agents. Gruhzit (2) in 1935 obtained a similar result, and reported the trypanocidal index of mapharsen to be 18, of arsphenamine 14, and of neoarsphenamine 9.

The use of mapharsen in the treatment of vivax malaria both in natural and induced infections was reported by Goldman (3) in 1938. Later its clinical use was studied by Cleveland and Turvey (4) in 1939. However, Young and McLendon (5) in 1939 reported that in their experience this arsenical did not eradicate the parasites of malaria, although it relieved the symptoms. This is in accord with other reports (6), that arsenic preparations, such as arsphenamine and neoarsphenamine, relieve the symptoms of malaria temporarily.

Since an appraisal of the therapeutic efficacy of mapharsen has been made in syphilis (7), trypanosomiasis (2, 7, 8), and vivax malaria (3, 4), it seemed advisable to study its possible usefulness against other parasitic infections. For this reason, the amebacidal activity of mapharsen was tested in comparison with other agents of suggestive value against cultures of *Endamoeba histolytica* *in vitro*.

Since emetine (9), carbarsone (10), (4-carbamino-phenyl-arsonic acid) and vioform (11) (Iodo-chloroxyquinoline) exhibit amebacidal activity *in vitro* and *in vivo*, a comparison of these agents with mapharsen and seven other compounds was

made. Among these, meta-nitrobenzoic acid (12) has been reported to have some action as a trypanocide *in vivo* and promin (N,N'-Disodium dextrose-sulfonate of 4,4'-diaminodiphenylsulfone (Parke-Davis) against *Brucella melitensis* (13). Other agents tested were: 2,4-dihydroxyphenylarsonic acid (Parke-Davis); N,N'-disodium formaldehyde bisulfite-3,3'-diamino-4,4'-di( $\beta$ -hydroxyethoxy) arsenobenzene (Parke-Davis); 4,4'-di(propionylamino) diphenylsulfone (Eli Lilly); p-tolamidine hydrochloride (Parke-Davis); and 4,4'-diamidinostilbene (Merck and Co.). A complete evaluation of these drugs in the chemotherapy of amebiasis was not attempted.

Three strains of *E. histolytica*, two isolated from man and one from *Macacus rhesus*, were grown successfully on coagulated egg media at 37°-37.5° C., after the technic of Dobell and Laidlaw (14). Throughout this study, we have used coagulated whole egg as the solid constituent (on a slant about 4 cm. in length) and egg-white diluted with Ringer's solution<sup>3</sup> as the liquid portion (10 cc. in each tube). At the same time, a suitable amount of solid rice-starch was added to the cultures. Measured quantities of the chemicals were dissolved in the liquid portion and then sterilized by passage through a Seitz filter. The pH of the liquid was adjusted to lie between 7.4 and 7.6 by the addition of a sterile solution of sodium bicarbonate. The amebacidal activity at various dilutions was observed at the end of twenty-four and forty-eight hours. Direct microscopic examination was made of a sample of the culture removed by the use of a capillary pipette from the surface of the culture slant.

A general summary of the data obtained on the amebacidal activity *in vitro* of the final concentrations of the various agents which were within the effective range appears in table 1.

<sup>1</sup> The chemicals used in this study were kindly supplied by the manufacturers.

<sup>2</sup> Research Fellow in Pharmacology.

<sup>3</sup> Dobell's modified Ringer's solution contains NaCl 9.0 gm., CaCl<sub>2</sub> 0.2 gm., and KCl 0.2 gm. Distilled water to make 1000 cc.



TABLE 1

*Amebocidal activity in vitro of arsenical and other compounds in comparison with known amebicides*

DRUGS USED	DRUG CON- CENTRATION IN VITRO	ENDAMOeba HISTOLYTICA					
		From man		From man		From macaque	
		38th, 42nd, 44th, 48th, 48th, 49th, 52nd, 54th, 57th, and 65th subcultures		18th, 27th, 30th, 34th, 35th, 38th, 40th, 43rd, and 51st subcultures		25th, 32nd, 35th, 39th, 40th, 43rd, 45th, 48th, and 56th subcultures	
		24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
3-amino-4-hydroxyphenyl-arsine oxide hydrochloride (maph- arsen)	1:15,000	—	—	—	—	—	—
	1:20,000	—	—	—, +	—	—	—
	1:30,000	+	—, +	+	—	—	—
2,4-dihydroxyphenyl-arsonic acid	1:6,000	—, +	—	—, +	—	—, +	—
	1:8,000	—	—	+	—, +	—, +	—
	1:10,000	+, ++	+	+	—	—, +	—
	1:12,000	++, ++++	+	+, ++++	+	+, ++	—
N,N'-disodiumformaldehyde bisulfite-3,3'-diamino-4,4'- di(β hydroxyethoxy) arseno- benzene	1:2,000	—, +	—	—	—	—	—
	1:3,000	+	—, +	—	—	—	—
	1:4,000	—, +	—	—, +	—, +	+, ++	—
	1:6,000	++	+	+	+	+, ++	—, +
Meta-nitro-benzoic acid	1:1,000	++	++	++	++	+	—, +
N,N'-disodium dextrose-sulfo- nate of 4,4'-diaminodiphenyl- sulfone (promin)	1:1,000	+++	++	++, ++++	++, ++++	++, ++++	++
4,4'-di(propionylamino)di- phenylsulfone*	1:5,000	++, ++++	++	++++	+	++, ++++	++
p-tolamidine hydrochloride	1:1,000	+++	++, ++++	++, ++++	++	++, ++++	+, ++
4,4'-diamidino-stilbene*	1:5,000	+++	+, ++	++, ++++	+, ++	+++	+, ++++
Iodochloroxyquinoline hydro- chloride (vioform-soluble)	1:6,000	+	—	+, ++	—	—, +	—
	1:8,000	++	+, ++	++	+	+	—
4-carbaminophenylarsonic acid (carbarsone)	1:4,000	++	—	—	—	—	—
Emetine hydrochloride	1:5,000	+	—	++	—	—	—
	1:10,000	++	+	++	+	—	—
Controls (18 tubes, each strain)	No drug	++ (3X) +++ (15X)	+ (4X) ++ (10X) +++ (4X)	++ (4X) +++ (14X)	+ (5X) ++ (10X) +++ (3X)	++ (9X) +++ (9X)	+ (2X)† ++ (15X)† +++

+++ = Vigorous growth; ++ = less vigorous growth; + = slight growth; — = no growth.

\* Not soluble in greater concentration.

† The number of times cultures were examined; for example, (2X) = an average of two times, (15X) = an average of fifteen times, and so on.

The amebacidal concentration of vioform-soluble is 1 to 6,000 on *E. histolytica-hominis* and 1 to 8,000 on *E. histolytica macacorum* at 48 hours. This is a somewhat higher concentration than observed previously by one of us (11). The present test was conducted in Dobell's and Laidlaw's coagulated egg medium instead of the Musgrave-Clegg's medium previously used and possibly there was less absorption of the drug by the medium. The findings reported with ameba isolated from man, which differed from the previous report, may have been due to the use of a more resistant strain of *E. histolytica hominis* isolated from a "cyst passer" who had been treated extensively with emetine hydrochloride 6 months before the culture was made. Against this strain as well as against the other strain tested, the amebacidal concentration of emetine hydrochloride was greater than the 1 to 25,000 dilution reported by Dobell and Laidlaw (9). They also observed, however, that *E. histolytica* might live and remain active for many hours in a 1 in 1,000 concentration of this alkaloid and it was thought that with a longer exposure, the drug was more effective. The amebacidal concentration of mapharsen was found to be 1 to 20,000 against one strain of *E. histolytica* from man at the end of 48 hours exposure and 1 to 30,000 dilution on the other strain from man and on the monkey strain. Other agents tested were less effective.

## SUMMARY

A study of the amebacidal activity *in vitro* of mapharsen and seven other compounds and three known amebicides is reported. Of the group of agents tested *in vitro*, mapharsen was most effective and killed *E. histolytica* in 1 to 20,000 to 1 to 30,000 dilution on 48 hours exposure. 2,4-dihydroxyphenylarsonic acid was effective in 1 to 8,000 dilutions against one strain of *E. histolytica* and N,N'-disodiumformaldehyde bisulfite-3,3'-diamino-4,4'-di( $\beta$ -hydroxyethoxy)arsenobenzene in 1 to 3,000 dilution was effective against two other strains. Other drugs had no appreciable effect on amebae in similar concentrations.

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# A PATHOLOGICAL STUDY OF THE ACUTE LESIONS PRODUCED BY PLASMODIUM LOPHURAE IN YOUNG WHITE PEKIN DUCKS<sup>1</sup>

R. H. RIGDON, M.D.<sup>2</sup>

*From the Department of Pathology, University of Tennessee College of Medicine, Memphis, Tennessee*

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Studies on fatal *P. falciparum* infections in man, and on *P. knowlesi* infections in monkeys suggest strongly that death from acute malaria is precipitated largely by the anemia and is accompanied by heart failure resulting from myocardial anoxia, (1, 2). The inoculation of ducklings with two billion *P. lophurae* parasites per kilogram body weight results in the development of almost a uniformly fatal infection. (3) In this respect these infections are similar to untreated *falciparum* and *knowlesi* injections.

A considerable portion of the voluminous literature on avian malariology is concerned with the host-parasite relationships. During the past fifty years observations have been made on some of the pathological changes occurring in avian malaria. It appears, however, from a review of the literature that a complete pathological study is indicated. In this paper the early pathological lesions produced by *P. lophurae* in young white Pekin ducks are reported.

## METHODS AND MATERIALS

The ducklings used were of the white Pekin variety and were two weeks' old at the time of inoculation. The inoculum consisted of blood obtained from ducks with *P. lophurae* infections, mixed with an equal volume of 2 per cent sodium citrate in physiological saline. The inoculum was injected into the leg vein, each bird receiving 2 billion parasites per kilogram of body weight. Subsequently the ducklings were killed at intervals of from 2 to 15 days (table 1). A complete autopsy was made immediately and tissues for histological study were placed into either one or all of the following fixatives: Carnoy's, Zenker-formalin, and 10 per cent solution of formalin.

<sup>1</sup> The studies on which this paper is based were aided by a grant from the Tennessee Valley Authority through the Division of Preventive Medicine, University of Tennessee College of Medicine.

<sup>2</sup> Department of Pathology, School of Medicine, University of Arkansas, Little Rock, Arkansas.

Paraffin sections were prepared from tissues fixed in the Carnoy's and Zenker's solutions and were stained routinely with hematoxylin and eosin. Tissues from some of the ducks were stained with Scarlet R for fat, by the Giemse technique to demonstrate parasites, and with acidified potassium ferrocyanide for the demonstration of hemosiderin (Prussian blue reaction).

TABLE 1  
*Ducklings used for pathological study*

NUMBER OF DUCK	INTERVAL BETWEEN INJECTION AND DEATH	DEATH		AVERAGE SIZE OF SPLEEN*
		Killed	Died	
	days			mm.
46, 47, 48, 240, 272	2	5	0	10 x 8 x 5
21, 22, 24	3	3	0	10 x 8 x 5
12, 13, 14, 15, 16, 19	4	6	0	23 x 16 x 10
27, 31, 32, 33, 34, 35	5	6	0	26 x 21 x 14
49	6	0	1	22 x 13 x 10
50	9	0	1	14 x 12 x 6
28	9	1	0	20 x 18 x 10
29	11	1	0	20 x 18 x 10
30	12	1	0	20 x 18 x 10
136, 146, 147	15	3	0	24 x 17 x 12
37, 41, 51, 52	Controls	4	0	14 x 9 x 7

\* These data are too few to be significant. They only indicate the trend of splenic enlargement.

## MACROSCOPIC PATHOLOGY

No pathological lesions were observed in birds killed on the second day following inoculation. Spleens from ducklings killed on the fourth day after inoculation were slightly, but definitely, larger than normal. From this time to the fifteenth day the spleen increased in size (table 1) and in pigmentation. The consistency of the larger spleens was relatively firm.

It is questionable whether the liver was pigmented on the second day; however, on the fourth day it was definitely pigmented. The degree of pigmen-

tation gradually increased to the fifteenth day. The lungs and the femoral bone marrow became progressively pigmented as the infections developed. Focal, reddish-blue areas, resembling hemorrhages, occurred in the lungs of some of the birds.

An acute fibrinous pericarditis accompanied frequently by hemorrhage in the epicardium occurred in several instances, most often after the fourth day following the inoculation. The larger hemorrhagic areas measured 0.5 cm. in diameter. The myocardium became progressively more pale and flabby during the acute phase of the infection.

in the splenic pulp. It was impossible to determine the exact location of the pigment in relation to the sinuses and the pulp. Nucleated red blood cells were found diffusely infiltrating the pulp at this time. As the infections progressed, the number of yellow granules of pigment increased in the spleen, and accompanying this increase the small granules apparently adhered to each other to produce small masses of pigment. These masses were distributed throughout the splenic pulp during the first six to seven days of the disease. After this time there appeared to be a tendency for these masses of

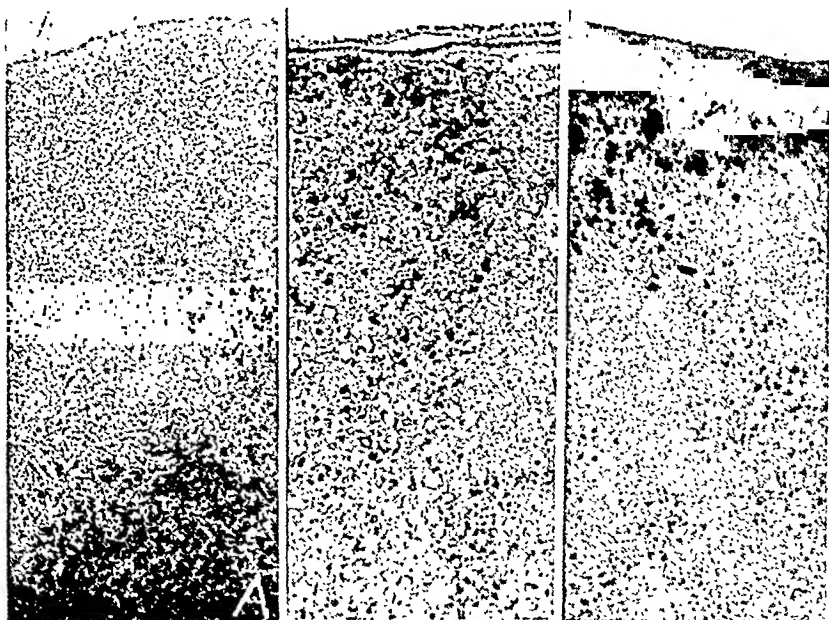


FIG. 1A. Red blood cells diffusely infiltrate the splenic pulp. The amount of pigment is insignificant. Duck 24 killed three days following inoculation.

B. The clear spaces in the pulp were formerly filled with red cells. Note the large masses of pigment. Duck 27 killed five days following inoculation.

C. The splenic pulp is filled with red blood cells. Masses of pigment are present. There is also a hyperplasia of the splenic pulp cells. Duck 29 killed eleven days following inoculation. H & E.  $\times 96$ .

In birds which survived, the color of the myocardium gradually returned to normal after the infection subsided.

No lesions were observed in the brain. There was an insignificant amount of pigmentation of the muscles, skin and parenchymal tissues other than those described previously; otherwise, these tissues showed little, if any, variation from the normal.

#### MICROSCOPIC PATHOLOGY

*Spleen:* On the second day of the infections a few small granules of malarial pigment were present

pigment to accumulate around the walls of the larger sinuses in the spleen.

There was an obvious decrease in the number of erythrocytes in the splenic pulp of some of the ducks. The pulp was filled with red blood cells in the normal ducks and during the first two to three days of the infection (figure 1A). After this time numerous spaces occurred in the splenic pulp which were formerly filled with red cells (figure 1B). Both the granules and the masses of pigment were more conspicuous in the ducks with the maximum infection. The splenic pulp again became filled with red blood cells in the birds that survived for

ten to fifteen days (figure 1C). In these latter ducks there was a hyperplasia of the reticular cells in the spleen.

Hyperplasia of the blood forming tissue in the spleen was most conspicuous in the walls of the larger venous sinuses. Masses of these cells extended into the lumen of the sinuses and a single layer of endothelial cells sometimes covered these groups of reticular-like cells (figure 2). Similar changes occurred also in the liver and the kidneys. Apparently these lesions are significant in the production of the vascular changes described subsequently.

The Scarlet R stains on the sections of spleen showed a large amount of lipoid granules within

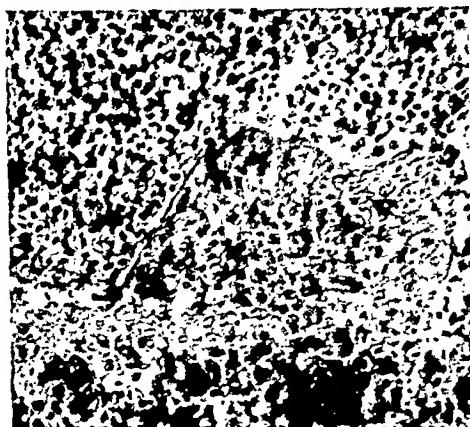


FIG. 2. The wall of a large venous sinus in the spleen. Cells like those in the splenic pulp project into the lumen of this vessel. These cells are covered by a layer of endothelium Duck 30, H & E.  $\times 368$ .

the cytoplasm of the phagocytic cells in the pulp. Malarial pigment and lipoid granules were present in the same phagocytes. Some of these phagocytic cells showed a predominance of either fat or malarial pigment. Lipoid granules likewise were present in the masses of hyperplastic cells in the wall of the venous sinuses. No hemosiderin was demonstrable in either the spleen or in any tissues of the ducks.

**Liver:** There was a progressive increase in the amount of phagocytosis by the K  pfer cells beginning about the second day of the infection. Pigment and cellular debris were present in the cytoplasm of these phagocytic cells. After the third day, vacuolation of the cytoplasm of the hepatic cells occurred in most of the birds. The cells showing the greatest amount of fat were located around the central veins. Necrosis of the hepatic cells sometimes occurred. In several

instances white blood cells were present in these areas of degeneration (figure 3). The necrosis in the hepatic lobule apparently always occurred around the central vein, sometimes it involved the greater portion of the lobule. The hepatic cells in such areas contained a large quantity of fat as shown by Scarlet R staining.

As the disease progressed there was an increase in production of blood forming tissue in the liver of these ducks. The degree of hyperplasia was marked after the fourth or fifth day of the disease. These cells were located primarily around the portal triads. The walls of the vessels here were infiltrated with young blood forming cells, groups of these cells projecting into the lumina. The size of the masses of these proliferating cells varied, as shown

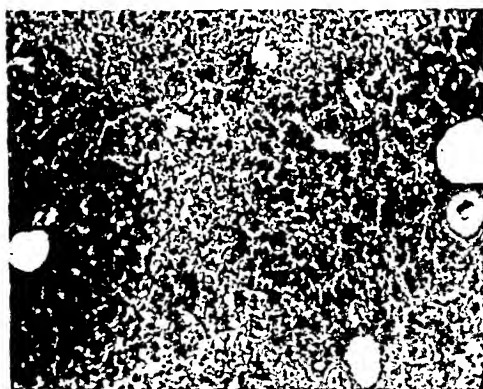


FIG. 3. Extensive necrosis is present in the liver of some of the ducks. This lesion occurs around the central vein of the hepatic lobule. H & E.  $\times 96$ .

in figure 4A. Sometimes the lumen of a hepatic vein was partially occluded. Frequently the surface of these masses of cells was covered by a pink-staining, fibrin-like material. Red blood cells and leucocytes sometimes adhered to the surface of these masses (figure 4B). Granules of malarial pigment were present both on the surface and in these groups of reticular-like cells.

**Kidney:** There was a varying amount of parenchymatous degeneration in the epithelial cells of the renal tubules. Sometimes the cells showed cloudy swelling, colloidal droplets and vacuoles of fat. Albuminous precipitate was present in the lumina of some of the tubules. No specific changes were observed in the glomeruli.

Masses of blood-forming cells were present in the medulla. These occurred in the walls of the blood sinuses and projected into the lumina in a manner similar to that described in the liver and in the spleen. The lumens of many of the

large blood vessels in some of the ducks were filled with these thrombus-like masses (figure 5).



FIG. 4. Masses of cells are attached to the wall and extending into the lumen of some of the larger hepatic veins. The cells in these masses are identical with those frequently found in the wall of these vessels. Sometimes pink staining fibrin-like material is present in these groups of cells. Leucocytes and red blood cells sometimes adhere to the surface of these masses as shown in figure B. Duck 29, H & E, A,  $\times 96$ ; B,  $\times 736$ .

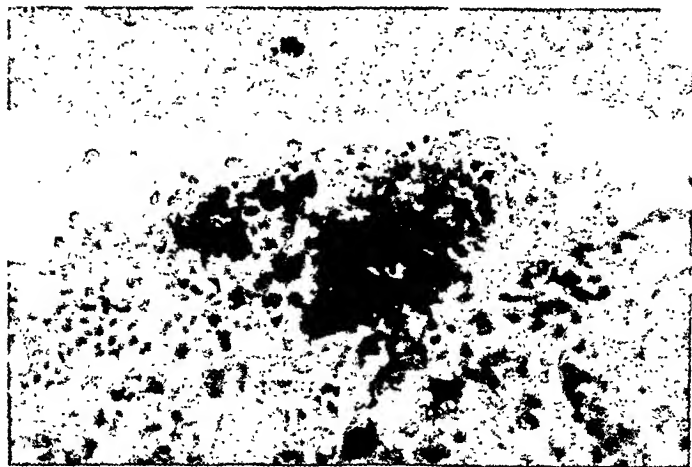


FIG. 5. Groups of cells project into the lumen of the venous sinuses in the kidney similar to those in the liver and spleen. Malarial pigment may be present in these groups of cells. Duck 29, H & E,  $\times 368$ .

**Lungs:** Granules and small masses of malarial pigment occurred in the lungs after the second day of the infection. The quantity of pigment increased progressively during the period of several days. This pigment appeared to be held by the circulating phagocytes in the lumina of the capillaries. As the disease progressed it

became increasingly difficult to demonstrate phagocytic cells in the larger masses of pigment. Phagocytosis of pigment by the endothelial cells in the blood vessel walls apparently was an insignificant process in these ducks. The pigment appeared to be primarily in the circulating phagocytic leucocytes. It is difficult, however, to be certain about the location of these phagocytic cells. As shown in figure 6, the pigment is retained in the lungs primarily within the lumina of the blood vessels. Sometimes masses of pigment were found apparently occluding the lumina of the capillaries in the interstitial tissues. Pigment was present infrequently within the alveolae.

Groups of red blood cells filled the lumina of some of the alveolae and similar cells were present in the lumina of the larger bronchi. These cells may result from strangulation at the time of killing the ducks. A few erythrocytes and edema fluid were present in the alveolae of the lungs of one duck that died spontaneously on the sixth day following inoculation. The pulmonary findings in this bird suggest that some of the blood observed in the alveolae in the others may be due to the infection.

**Blood vessels:** One of the most interesting of all the pathological lesions in these ducks

occurred in the blood vessels. Groups of mononuclear cells were present in the lumina of some of the large blood vessels after the fifth day of the disease (figure 7). The groups of cells were similar to those in the wall of the blood vessels in the liver, the spleen, and the kidney, and appeared to be hemopoietic cells. Leucocytes,

red blood cells, and malarial pigment were sometimes found in association with these masses of cells. This variation also occurred in the thrombotic masses described in the walls of the sinuses in the parenchymatous organs.

The lumina of many of the vessels throughout all the tissues were filled with red blood cells. No abnormal distribution of the cellular elements was observed in any of the vessels. The endothelial cells lining the vascular system was carefully studied for phagocytosis. During the first six to eight days of the infection it was difficult to determine whether some of the masses of pigment were present either in the circulating phagocytes or in the endothelial cells. In a very few instances malarial pigment was found

served in the endothelial cells in the cerebral vessels.

There was a variation in the amount of fat in the cerebral vessels of the different birds. Duck 49 died on the 6th day, and there was a diffuse distribution of lipoid in its capillaries. Duck 30 survived the acute infection and was killed on the 12th day of the disease. Only an occasional endothelial cell with fat was found in this bird.

*Heart:* The ducks with an acute pericarditis had red blood cells, leucocytes, edema, fibrin, and young myeloid-like cells infiltrating the epicardium. This cellular reaction sometimes

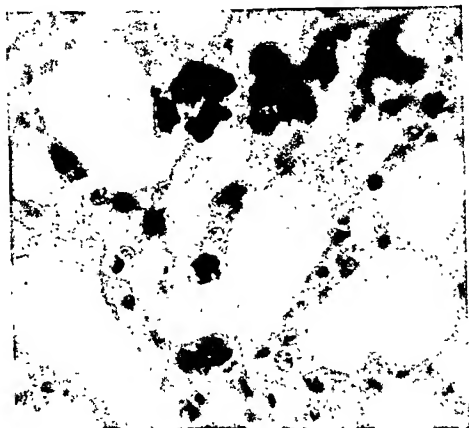


FIG. 6. Masses of malarial pigments are present in the capillaries in the interstitial tissue of the lungs. Infrequently malarial pigment is present in the alveolae. Duck 29, H & E.  $\times 736$ .

within the cytoplasm of endothelial cells. Phagocytosis by these endothelial cells was observed more frequently in the vessels of the birds that survived the infection for the longest periods of time.

The endothelial cells lining the small blood vessels in the brain were studied carefully in both the hemotoxylin and eosin stains on paraffin sections, and the Scarlet R stains on the frozen sections. Lipoid masses were present in the endothelial cells of these vessels. The globules were usually small. Sometimes masses of malarial pigment and globules of fat were present in the same cell. It was difficult to ascertain beyond question whether this fat and pigment were always in either the endothelial cells or in the circulating phagocytes. In the hemotoxylin and eosin stains malarial pigment was not ob-

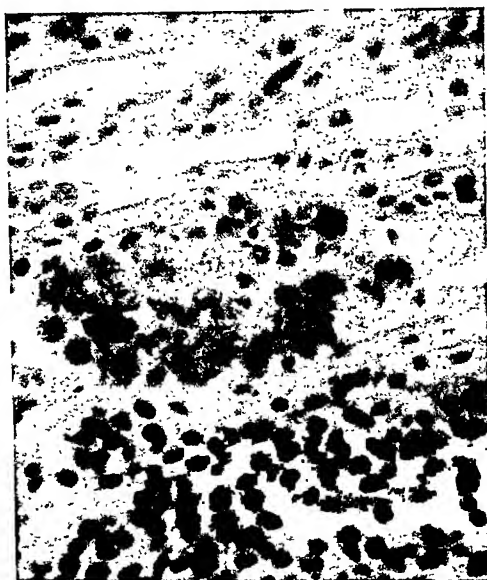


FIG. 7. Groups of large reticular like cells are present in the lumen of some of the larger blood vessels in the lungs. These cells are similar to those occurring in wall of the veins in the liver, spleen, and kidneys. Duck 29, H & E,  $\times 736$ .

extended down into the cardiac muscle. Focal areas of degeneration were present in the myocardium of some of the ducks and these were infiltrated with red blood cells and leucocytes. These masses of cells sometimes resembled groups of extramedullary blood-forming tissue.

Sections of the myocardium stained with Scarlet R showed a few mononuclear phagocytic cells with their cytoplasm filled with lipoid granules. These cells occurred more frequently about the periphery of large blood vessels. Very infrequently lipoid material was found in the cytoplasm of the endothelial cells lining the blood vessels. The lumen of some of the small blood vessels were filled with globules of lipoid



which stained with Scarlet R. Sometimes the fat in such vessels appeared to be present both within the circulating phagocytic cells and in the lining endothelial cells. In other instances the globules appeared in clusters filling the lumen of the vessels. Masses of lipid granules occurred in the myocardium of normal ducks, the location being similar to that described for the infected birds; however in the latter the process was more pronounced.

**Bone marrow:** It was impossible to determine the degree of hyperplasia present in this small group of ducks. Since the birds were quite young the marrow was normally very cellular. The histological studies made upon only a few of these birds suggested that hyperplasia was present. Phagocytic cells in the marrow were filled with malarial pigment, but there was wide variation in the degree of phagocytosis in the various ducks.

**Brain:** No hemorrhages were found in the brains of the ducks. A detailed study of the neurological lesions in these ducks will be included with those in man and monkey to be published in a separate paper.

**Intestinal tract:** A section of the intestines was removed from each bird and no pathological changes were observed.

**Muscle:** No pathological changes were observed in the striated muscles.

#### DISCUSSION

The basic pathological lesions occurring in these ducks are similar to those previously described in a case of *P. falciparum* in a child and *P. knowlesi* in *Macacus rhesus* monkeys (1, 2). There is a marked decrease in the number of red blood cells observed in the spleen of the ducks that either die or are moribund when killed. The pale color of all the viscera is indicative of a severe anemia. Clinical observations confirm the development of a severe and rapidly progressing anemia in young ducks infected with *P. lophurae* (3).

In the previous studies referred to, the relationship of pulmonary edema and central necrosis in the liver to a failing myocardium has been discussed (1, 2). Each of these lesions may develop when the myocardium becomes anoxic. In the presence of a marked decrease in the number of red blood cells, and the remaining cells being highly parasitized, it would seem most likely that the transportation of oxygen

and the removal of waste products would be markedly affected. An increase in capillary permeability is one manifestation of anoxia (4). The concentration of red blood cells within the lumina of the blood vessels would suggest that fluids probably escaped into the extravascular tissues. It is, of course, difficult to demonstrate pathologically small amounts of edema within the tissues. The edema fluid in the lungs may be explained on the basis of anoxia. Lipoid granules in the endothelial cells of the brain likewise indicate injury which may have resulted from anoxia.

The presence of groups of cells within the lumen of the larger blood vessels in these ducks must not be confused with the groups of agglutinated red blood cells described by Knisely *et al.* in monkeys infected with *P. knowlesi* (5). The groups of cells present within the lumen of the blood vessels of these ducks are similar to the hyperplastic cells occurring within the walls of the blood vessels in the liver, the kidney, and the spleen. It is significant that these groups of cells are found only following the process of hyperplasia. These cells have the histological characteristics of extramedullary blood forming tissue. It is important to remember that extramedullary blood formation is conspicuous in young ducks. Blood forming tissue occurs in virtually every normal organ of the body, being especially pronounced in the lungs, liver, and in the spleen. A hyperplasia of this type of tissue may occur in leukemia (6). The clumps of cells as observed by Knisely are present much earlier in the course of the infection in the monkey. The clumps of cells described by Lack in the blood vessels located on the inner surface of the wings of canaries infected with *P. cathemerium* likewise occur at a much earlier period in the disease than the masses of cells observed in the ducks (7).

Groups of cells resembling those in the splenic pulp are present in the wall of the large venous sinuses in premature infants. Similar cells have been observed in a child's spleen infected with *P. falciparum* and also in the spleen of monkeys injected with *P. knowlesi* (2). In no previous pathological study, however, has the writer observed the extensive hyperplasia that is present in some of the ducks infected with *P. lophurae*.

It appears very likely that hyperplasia of the extramedullary tissue in the walls of some of the larger venous sinuses may be an important

factor in the development of splenic infarcts. Observations are being made now, and will be reported in a subsequent publication, on the spleen from man, monkey, and bird with malaria in an effort to determine the significance of this process.

Bruetsch (8) has described vascular lesions in the spleen of human cases of *P. vivax* malaria similar to these in the duck. He states that, "The endothelium of larger trabecular veins was frequently lifted up by an accumulation of plasma cells, basophilic round cells, and lymphocytes. Within the trabecular veins there was a great number of macrophages, plasma cells, basophilic round cells, and lymphocytes. Sometimes large groups consisting of 25 to 50 macrophagic reticular cells were present in the lumen of the veins. They had the tendency to adhere to each other entangling other white cells and red corpuscles in their meshes. Occasionally, an elongate, entirely unstimulated reticular cell was found floating within the trabecular veins."

It is especially interesting to find in the ducks a few cells lining blood vessels that contain malarial pigment. Phagocytosis by similar cells was not observed in the monkeys infected with *P. knowlesi* or a child with *P. falciparum*. The infrequency of phagocytosis by endothelial cells in the duck would indicate, however, that such cells do not play a significant role in the process of phagocytosis of malarial pigment and parasites. Phagocytosis occurs primarily in the Küpffer cells of the liver and in the spleen. Which cells are phagocytic in the spleen is not determined by this study. A small amount of phagocytosis also occurs in the bone marrow.

The absence of hemorrhages in the brain of these ducks is important in considering the mechanism of hemorrhages in the human brain in case of *P. falciparum* infection. The degree of parasitization of ducks with *P. lophurae* is much greater than that observed in man with *P. falciparum*. The possibility of clumps of these cells plugging the cerebral vessels likewise would appear to be more likely in the duck than in the human brain. In view of the lesions observed in the human brain and in the brain of the monkeys, and in the absence of a corresponding lesion in ducks infected with an

acute type of malaria, it is suggested that obstruction in the cerebral vessels by masses of parasitized red cells may not be the entire explanation for the hemorrhages that occur.

The pathological lesions, observed in these ducks killed within the first fifteen days of the infection with *P. lophurae*, suggest that the rapidly progressing anemia plays a most significant role in the production of death. The anemia, of course, results from the rapid destruction of the red blood cells by the parasites. The extensive hyperplasia of the blood forming tissues is indicative of the reaction of the host to the anemia.

#### SUMMARY

The pathological lesions in young ducks infected with *P. lophurae* are described. It appears from this study that the rapid development of an anemia, resulting from the destruction of the red blood cells by the parasites plays a significant role in the production of these lesions.

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# UNUSUAL BREEDING PLACES OF MOSQUITOES IN THE VICINITY OF KEESLER FIELD, MISSISSIPPI

FRANK N. YOUNG, JR.<sup>1</sup> AND WARREN N. CHRISTOPHER<sup>2</sup>

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During the 1943 season, several species of mosquitoes have been observed breeding in unusual situations at Keesler Field, Mississippi and in the nearby town of Biloxi. Few of the situations observed were producing mosquitoes in large numbers, but they emphasize the difference between habitat preference and habitat tolerance, and the danger of stereotyped thinking with regard to breeding places.

*Anopheles quadrimaculatus* Say. This species was fairly abundant at Keesler Field and in the vicinity during June and July. The region presents no typical habitats such as one finds in the highly malarious regions of the Southeast, but the mosquito is apparently able to adapt itself to a variety of breeding places which would not ordinarily be considered favorable.

At one point on Keesler Field, larvae were found in some numbers (8, 4th instar, July 13; 1 (reared) July 27; 8, 4th instar, July 19), in tiny pools in a running ditch. The ditch, cut through a seepage area in the old Naval Reserve Park, at no time except during heavy rains carried more than an inch of water.

An unusual number of adults in certain resting stations initiated an intensive search which revealed the species breeding abundantly (10, 4th instar, July 15; 6, 4th instar, July 16; 9 (reared) July 20. Small larvae and *crucians* plus *quadrimaculatus* 12.4/dip.) in about two inches of water flowing from a broken water main, and flooding the grassy area between a curbing and the sidewalk.

In the city of Biloxi, 2, 4th instar larvae were collected July 15, along with *A. crucians* breeding in a small ditch fed by a spigot left running constantly at about half force to supply water for a

drinking trough. At another point, 1, 4th instar larva was taken July 16 in a similar, but much smaller situation where the faucet was allowed to drop for the benefit of chickens.

*Anopheles punctipennis* (Say). Three, 4th instar larvae of this species were collected May 18 from a firebarrel at Keesler Field.

*Anopheles crucians* Wied. Three, 4th instar larvae of typical *A. crucians crucians* were collected August 20 from a large tin can on the edge of a small marsh in Biloxi. The can was quite rusty, but the water was clear and considerable leaf debris had collected at the bottom.

*Aedes taeniorhynchus* (Wied). This species was taken breeding in fresh water at two stations on Keesler Field. (1, August 30; 2, September 8).

*Aedes sollicitans* (Walk.) ? *Aedes* larvae which are very similar to typical *sollicitans* except in the great development of the anal gills were collected in some numbers during September in several fresh water habitats on Keesler Field. Most of these were identified by the authors as *Aedes mitchellae*, but the entomologist at Gulfport Field has found similar larvae which were reared and proved to be *sollicitans*.

*Culex nigripalpus* Theob. Two larvae of this species were taken September 9 from brackish pools, on a salt marsh at the edge of Keesler Field, where *Aedes taeniorhynchus*, *A. sollicitans*, and *Anopheles bradleyi* were breeding abundantly and *Culex salinarius* was rarely found. There seem to be no previous records of this species breeding in brackish water.

*Aedes triseriatus* (Say). This species breed abundantly in water in old tin cans during March, April and May at the can dump at Keesler Field.

*Orthopodomyia signifera* (Coq.). Larvae were found breeding in water in tin cans along with *A. triseriatus* and *Culex quinquefasciatus* on July 22.

<sup>1</sup> Second Lieutenant, Sanitary Corps, A.U.S.

<sup>2</sup> Major, Sanitary Corps, A.U.S.



# CULTIVATION OF LEISHMANIA IN THE YOLK SAC OF THE DEVELOPING CHICK EMBRYO

HELEN JONES, GEOFFREY RAKE AND DOROTHY HAMRE

*From the Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick, N. J.*

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Geiman (1) has described briefly the cultivation of strains of *Leishmania* in the allantoic fluid of the developing chick embryo. Cultures of *L. tropica* were carried for 26 passages but *L. braziliensis* only survived for 2 passages. No tissue or blood stream invasion occurred with any strains. The yolk sac method of inoculation of the developing chick embryo, first developed by Cox (2), has been used in our hands for the study of several virus-like agents (3-5) and bacteria (e.g., *H. Ducreyi* (6)). Because of the difficulty of maintaining strains of *Leishmania* in this country by any method than that of blood agar culture, which gives only the leptomonad form, it seemed of interest to determine whether this group of protozoa could be cultivated by the simple yolk sac technique. Three strains of *Leishmania* were used (a) *L. donovani* (?) of unknown origin obtained from Dr. M. Kahn; (b) *L. tropica* obtained originally from Dr. H. A. Senekji; (c) *L. braziliensis* obtained originally from Dr. O. da Fonseca. All were made available to us through the kindness of Dr. Roy Greep. They had all proven non-pathogenic in animals.

## TECHNIQUE

Chick embryos of 6, 7, 8 or 9 days prior incubation were used throughout. There was no evidence of difference in susceptibility within this age range. 1 ml. volumes of inoculum were used in all cases, previous experience having shown that this volume, presumably because of better dissemination through the yolk, gives more consistent results than do smaller volumes. Three incubation temperatures were used, namely, room temperature (which fluctuated between 19°C. and 23°C.), 28°C. incubator and 36.5°C. incubator. The results obtained at these three levels will be discussed below. As far as the chick embryos themselves were concerned they developed almost normally at 36.5°C. and slowly but significantly at 28°C. At room temperature they survived but showed only very slight or no grossly observable development. All eggs which

died, or which were to be studied after a given period of incubation, were opened and emptied into sterile Petri dishes. A small piece of the yolk sac was smeared on clean glass slides. Such smears were fixed with methyl alcohol and stained with Giemsa's stain or fixed with heat and stained by Macchiavello's method. In those cases where the yolk smears showed infection with *Leishmania* and it was desired to use the material for passage, the yolk sac or yolk sacs were removed and shaken in their own pooled allantoic fluid and yolk in a volume equal to 10 times their weight. In titrations, further dilutions were made in pooled allantoic fluid and yolk from normal uninoculated eggs.

## RESULTS

In general it may be stated that all strains<sup>s</sup> showed some multiplication at all temperatures<sup>s</sup> of incubation tested. At 36.5°C. the organisms<sup>s</sup> apparently developed poorly in the case of all strains. Only a small proportion of the eggs died (26 per cent) and most of these died late (10 to 15 days after inoculation). However, when the embryos were opened even as late as the 21st day of incubation, and a piece of the yolk sac smeared and stained, the majority of eggs were found to be infected (75 per cent). At room temperature the results were satisfactory while this was approximately 23°C. 96 per cent of eggs died specifically and even in the early (1st to 5th) passages death occurred in 3, 4 or 5 days in 94 per cent, following yolk sac inoculation of a 10 per cent suspension of infected yolk sac in homologous yolk and fluids. When the temperature of the room fell to 21°C., and even to 19°C., most of the eggs still died (except in the case of the strain of *L. tropica*) but 63 per cent of the deaths did not occur before the 9th day. After 7 passages under conditions of fluctuating and comparatively low temperatures, all strains were carried in an incubator at 28°C. Mortality within a 3, 4 or 5 day period reappeared in 97 per cent of the eggs. It was, therefore, concluded that the optimal

temperature for the<sup>7</sup>cultivation of *Leishmania* in the yolk sac lies between 23°C. and 28°C.

#### *Leishmania donovani*

This strain has been carried in the yolk sac for 14 passages at 36.5°C. and for 31 passages at lower temperatures. It has proven almost uniformly lethal (95 per cent) and the smears of the yolk sacs have shown protozoa often in large numbers. Dilutions of 1/1000 of yolk sac or higher were not lethal. In the first few passages a few flagellates (leptomonad forms) were seen in smears from the embryo—specifically smears from liver or spleen—but later these were not observed. For the most part the protozoa seen in the yolk sac smears were in the leptomonad form, not infrequently indeed in the form of “agglutinated” rosettes, but occasionally there were forms which were definitely suggestive of Leishman-Donovan bodies, and there were also larger bizarre non-flagellated forms whose size and ovoid shape suggested degenerated leptomonads. When sections of the yolk sac and embryo were prepared the pathological picture became clearer. Protozoa were not seen in any organs of the embryo. They were plentiful however in the yolk cells of the yolk sac, often when the smears of these sacs had not shown very heavy infection, and might also be seen scattered or in groups or rosettes in whatever yolk had remained adherent in the section. While definite leptomonads and Leishman-Donovan (L-D) bodies could be found both in the yolk cells and in the yolk, L-D bodies were most numerous in the cells and were usually scanty in the overlying yolk. Rosettes of leptomonads or groups of leptomonads and L-D bodies were often seen in the yolk. In the yolk cells it was striking that a group of twenty or more neighboring cells might all be infected and that apparently all forms present would be leptomonads. In another group of infected cells the L-D form would predominate. Large areas free from infection would separate such infected groups. One has the impression that multiplication of both the L-D forms and the leptomonads occurs in the yolk cells but that either conversion to the leptomonad form or multiplication of this form, or both, occurs in the yolk.

#### *Leishmania braziliensis*

This strain has been carried for 14 yolk sac passages at room temperature or 28°C. It has proven

lethal in all eggs by this method and smears of the yolk sac have usually shown very heavy infestation. Dilutions of 1/125 of original yolk sac were infective. Higher dilutions have not been tested. The pathological picture resembled that described for *L. donovani*.

#### *Leishmania tropica*

This strain has also been carried through 14 yolk sac passages at room temperature or 28°C. Unlike the other two strains which were transferred very readily from blood agar to the yolk sac of the developing chick embryo and gave good infections from the first, this strain infected very poorly for the first four passages, infecting only 31 per cent of the eggs. From the fifth passage on, however, this strain behaved like the others, infecting all eggs and giving smears showing infestation approximately as heavy as *L. donovani*. On section of the yolk sac, fewer *Leishmania* were seen than with the other two strains but otherwise the picture was unaltered.

Yolk sac material from *L. donovani* (2nd passage at 38.5°C.) and from *L. braziliensis* (2nd, 5th, and 6th passages at room temperature) were given to Dr. Roy Greep of the Division of Pharmacology of the Squibb Institute who inoculated them into hamsters, but was unable to produce any infection in these animals. We wish to express our gratitude for this and other help given us by Dr. Greep in connection with this study.

#### SUMMARY

Three strains of *Leishmania* have been cultivated in the yolk sac of the developing chick embryo. One strain has been carried for 31 consecutive passages by this method. The protozoa infect the yolk cells and multiply there both as Leishman-Donovan bodies and as leptomonads, but they also appear to multiply in the yolk as leptomonads. The strains failed to infect hamsters before inoculation into eggs. A few serial passages in eggs, although it gave rise to L-D bodies which were not seen in the prior agar cultures, also failed to give any enhancement of infectivity for these animals. A simple method of cultivation of these protozoa, giving both flagellated and unflagellated forms, has been developed and offers promise for their maintenance in the laboratory and, perhaps, for use in chemotherapeutic studies.

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# AN IMPROVED METHOD FOR MOUNTING MOSQUITO LARVAE

JOHN F. WANAMAKER<sup>1</sup>

*From the Fourth Service Command Laboratory, Fort McPherson, Georgia*

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The importance of mosquito identification at army installations in this country and overseas has shown the need for a rapid permanent method of mounting mosquito larvae. At the Fourth Service Command Laboratory, the Entomology Department has had experience in mounting hundreds of larvae on micro-slides since its establishment. Many methods have been employed in preparing the slides and as difficulties were encountered, an effort was continually made for improvement. Slides are primarily prepared for teaching mosquito identification and for this purpose it is necessary to have permanent, durable mounts that retain all taxonomic characters. Also a comparative larval collection must be maintained to show clearly all characters, obscure or variable, that aid in the determination of closely related species.

Officer entomologists attending the two weeks course in Mosquito Identification were desirous of learning to make their own comparative collections to aid them in the identification of the local species at their respective stations. As these officers in time expected to be assigned to Malaria Survey or Control Units they were desirous of learning a method of mounting mosquito larvae that they could successfully use in overseas areas. This called for a rapid, simple method of mounting mosquito larvae; one that could be easily used even in a field laboratory, gave permanent results, and was unaffected by changes in moisture or temperature.

The usual method of clearing in xylene and mounting in balsam was found, in the very beginning, to be unsatisfactory. By this process, specimens were dehydrated by running them through progressively higher concentrations of alcohol. Then the inner tissue was cleared so that head hairs and other diagnostic characters on the larvae could be easily observed. After this processing, which took several hours, the specimens were mounted in Canada balsam. The disadvantage of this method was that the xylene, as a clearing

agent, made the larvae so brittle many hairs were broken as the specimens were arranged in the balsam.

The laboratory turned to another standard method, using chloral gum as a mounting medium. In this process the specimen had to be completely hydrated. While the mounting process was rapid, much time was actually required before a slide was ready for use. Drying took several weeks even in an incubator room. While the slide was drying it had to be frequently checked and more chloral gum added as evaporation took place. When completely dry, the edges of the mount had to be closed with some sealing medium. Unless the chloral gum was made exactly to formula, temperature changes caused the mounting medium to crystallize. Over a period of four to five years the chloral gum mount has a tendency to turn brown. However, for temporary mounts this medium has some advantages.

To overcome these difficulties, a search was made for a clearing agent that left the larvae soft and pliable. Creosote U.S.P. was successfully used as a substitute for xylene in the clearing process<sup>2</sup>, but it soon became apparent that further improvement was necessary. After clearing in the creosote U.S.P., specimens occasionally collapsed when placed in the Canada balsam, and dark specimens were seldom sufficiently cleared. Also, the process was so slow it was impossible to keep up with the demand for study slides.

After considerable experimentation, the following method has been developed at the Laboratory and has been used in preparing over 2300 larval slides all with equally excellent results. Most important, the actual time now required to prepare a slide has been very greatly reduced.

Larvae are preserved and stored at the Laboratory in 80% alcohol. For permanent mounting they are taken from the 80% alcohol and placed in 95% or absolute alcohol for a period of from one

<sup>2</sup> See *Science*, March 10, 1944, Vol. 99, A Rapid Method for Making Permanent Mounts of Mosquito Larvae, by Captain W. W. Middlekauff of the Fourth Service Command Laboratory.

<sup>1</sup> Sergeant, Medical Department A.U.S.

to ten minutes. The longer the specimen has to remain in the higher alcohols the more brittle it becomes and the more easily hairs are broken off. From the 95% or absolute alcohol<sup>3</sup> the specimen is transferred to a clean slide containing the mounting-clearing medium, by means of a small spatula made from a smooth bent end of an applicator stick. This mounting medium is composed of Canada balsam, which has been heated slowly to drive off almost all the xylene, and then thinned with the creosote U.S.P. to the desired consistency.

<sup>3</sup> Except for the most delicate specimens, larvae may be transferred from 80% alcohol directly to the creosote-balsam, with satisfactory results, thus further speeding up the mounting process.

It has been found that a large specimen is mounted more easily if the mounting medium is thicker than that normally used for a small thin specimen. After the larva has been properly arranged on the slide, a cover slip is immediately placed over it. After drying for 10 to 12 days, preferably in an incubator, the slide is ready for use.

Freshly collected specimens should be killed in hot water, and immediately transferred to 95% alcohol. After 5 minutes, carefully transfer the specimen to fresh alcohol and in another 10 minutes the specimens are ready to be placed on the slide. Any white liquid that might be emitted from the larvae after they are placed in the creosote-balsam, will disappear as the slide dries.

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